

October 25, 1989

Thomas M. DeLauro, DPM
Editor, Journal of the American
Podiatric Medical Association
9312 Old Georgetown Road
Bethesda, Maryland 20814-1621

Dear Tom:

I am submitting for your consideration our research paper entitled "Aloe Vera as a Biologically Active Vehicle for Hydrocortisone Acetate" for the 1989 William J. Stickel Awards.

As you are aware, we have previously shown that decolorized Aloe vera is active in wound healing, inflammation, and adjuvant arthritis. This study attempts to use Aloe vera with hydrocortisone acetate to improve the effectiveness of this type of therapy. Inflammation is a major component of many of the conditions treated by the podiatrist. The significance of this study lies in the ability to increase steroid potency by using a natural substance.

We continue our research with Aloe vera in hope of providing podiatric physicians with safe and effective alternatives to manage diseases of the lower extremity.

Should you have any questions about the work, please let me know.

Best wishes,

Sincerely,

Robert H. Davis

Robert H. Davis, Ph.D.

RHD/dpm
Enc.

ALOE VERA AS A BIOLOGICALLY ACTIVE VEHICLE
FOR HYDROCORTISONE ACETATE

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ABSTRACT

Aloe vera as a biologically active vehicle for hydrocortisone-21-acetate was tested against acute inflammation. A mustard-induced paw-swelling assay determined the systemic activity of Aloe vera and hydrocortisone acetate. The topical activity of these agents was evaluated with a croton oil-induced ear-swelling assay.

Systemically, the combination of Aloe vera and hydrocortisone produced a maximum 88.1% inhibition of edema. Polymorphonuclear leukocyte infiltration was reduced 91.1%. The topical inhibition of edema peaked at 97%. The possibility that Aloe vera has significant potential as a vehicle for steroids was discussed. An attempt was made to examine "how Aloe works" against a background of mediators and oxygen radicals in inflammation.

INTRODUCTION

Diseases characterized by inflammation are a major cause of morbidity and mortality in humans. Inflammation can best be described as a necessary and inter-related series of cellular and immune responses to tissue insult caused by physical trauma, microbial invasion or immune disorders. While vital to the healing process, inflammation, if uncontrolled, can lead to more serious conditions. Since the discovery of hydrocortisone in 1952, the efficacy of topical corticosteroids in inflammation management has been well established. Inflammation arising from irritants, surgery and severe arthritic conditions has been successfully treated with steroids. Topical corticosteroids are the largest single class of dermatologicals in sales commensurate with their importance to patients.¹

Corticosteroids decrease edema by reducing capillary permeability, vasodilation, and by stabilizing lysosomal membranes.² However, steroids can actually increase the spread of infection by inhibiting connective tissue formation.² Steroid effectiveness depends on dosage, hormone distribution, absorption rate and their influence on peripheral white blood cells. Since glucocorticoids have a poor rate of absorption through the skin, vehicles take on great importance to physicians to enable the steroid to get to the inflammation site.³ Should the vehicle have biological activity as well, then the physician has a strong tool to combat inflammation. We continue research to provide an agent that demonstrates anti-inflammatory and wound healing

activity without the detrimental side effects of the current synthetic treatments. Should Aloe vera have an additive or synergistic effect with hydrocortisone acetate on reducing inflammation, we would propose its use as a vehicle to reduce the dose of steroid to eliminate or lower the risk of side effects in treatment. This finding, we feel, would be a major contribution to podiatric medicine.

CORTICOSTEROID SIDE EFFECTS

The benefits of corticosteroid therapy must be viewed with the undesirable side effects in mind. These side effects relate to dosage of steroid, duration of therapy, surface area and condition.⁴ Localized effects observed include: increased skin irritation, excessive dryness, allergic reactions and increased spread of infection. The side effects associated with prolonged use and more potent steroids tend to be more severe and less localized. The more commonly seen systemic effects are; skin atrophy, permanent telengectasias (spider veins), glaucoma, iatrogenic Cushing's syndrome and hypothalamic-pituitary-adrenal axis suppression.^{4,5} These deleterious effects can be associated with both topical and systemic routes of administration.

Overapplying the steroid exacerbates toxic effects. Repeated daily application of steroids does not increase skin absorption, instead, studies show an increased tolerance and a reduced clinical effect.⁴ Clinical studies demonstrate that percutaneous absorption of steroids is minimal. In fact, only about 1% can penetrate the stratum corneum of the skin. Since the steroid can have no effect unless it is absorbed, this means that 99% is

unavailable and wasted.⁵ This is a significant economical consideration for expensive compounds like steroids which recommends the use of a more efficient carrier.

STEROID VEHICLES

Vehicle choice becomes paramount when using steroids or any drug. The vehicle dramatically impacts on the availability and efficacy of corticosteroids. Criteria for vehicle selection include: the solubility of the active agent, the ability of the vehicle to hydrate the stratum corneum and the stability of the active agent in the vehicle. Consideration must also be given to any interactions between the vehicle, active agent and the stratum corneum.⁶ Traditional vehicles can be classified as ointments, creams, lotions, powders and aerosols. Studies have demonstrated that ointments are most effective but creams and lotions, while less effective, are more acceptable to patients.^{7,8} Patients dislike therapy regimens whose vehicle feels greasy, looks unsightly or stings when applied. Such cosmetic considerations can effect patient compliance and the success of therapy. As a vehicle, Aloe vera may fulfill both pharmacological and cosmetic requirements.

ALOE VERA ACTIVITY

Aloe vera's anti-inflammatory, wound healing and analgesic properties have been supported through many case studies.⁹ Research of Aloe vera's biological activity was launched by the work of Collins and Collins, in 1935, on the treatment of roentgen dermatitis (x-ray burns).¹⁰ Aloe vera research has taken two main directions. Firstly, to seek for its active

ingredients, and secondly its overall effects. The past 20 years has shown an increased interest in the search for Aloe vera's active ingredients. Aloe vera is a succulent leaf comprised primarily of water (1:200). In addition, Aloe vera's broad categories of ingredients include: lignin, saponins, anthraquinones, inorganic materials, minerals, vitamins, mono and polysaccharides, enzymes, amino acids (essential and secondary) and gibberellin.¹¹ Davis has shown that gibberellin can account for a major part of the biological activity (60.1%) when measured against gelatin-induced inflammation in diabetic mice.¹² In fact, the dose-response relationship closely paralleled the Aloe vera alone.

The Aloe vera gel consistently inhibits infection and promotes healthy tissue growth.¹¹ Decolorized Aloe vera reduces edema and inflammation in normal rats and in the diabetic model was effective in healing wounds, edema and pain.^{13,14} This laboratory demonstrated topical and systemic activity of Aloe vera in the prevention and inhibition of adjuvant-induced arthritis.¹⁵ Aloe vera blocked irritants such as gelatin, kaolin, carageenin, mustard and croton oil.¹⁶ Davis showed that Aloe vera inhibited inflammation but, also improved wound healing.¹⁴ Since steroids inhibit wound healing, Aloe vera's positive influence on wounds and circulation make it a natural as a vehicle for corticosteroids. The irritant-induced paw-swelling assay has been used as an indicator of systemic anti-inflammatory activity for many years.¹⁷ Topical anti-inflammatory activity can be evaluated using an ear-swelling assay in the mouse.¹⁸

Both of these techniques are standard models for inflammation research. The quantification of polymorphonuclear leukocyte infiltration into a site of tissue damage is a valuable tool for evaluating the acute anti-inflammatory activity of many compounds including steroids and Aloe vera.¹⁹ PMN infiltration is closely linked to the theory of oxygen radical formation in inflammation.²⁰ The oxygen radical theory provides insight into the mechanisms of inflammation and can be used to understand the activity of Aloe vera which may act as an oxygen radical scavenger.

PURPOSE OF STUDY

This work proposes to compare the topical and systemic anti-inflammatory activity of hydrocortisone acetate with Aloe vera. We attempt to determine if the biological activity of both agents are at least additive so that Aloe vera can be used as a vehicle for hydrocortisone acetate. Possibly, Aloe could aid the absorption of the steroid so that the steroid dosage could be reduced without losing treatment effectiveness. PMN infiltration, paw edema and ear-swelling will be used as endpoints in an attempt to explain "how Aloe vera works". Consideration will be given to mechanisms of action.

MATERIALS AND METHODS

The topical influence of decolorized Aloe vera (without anthraquinones) on the anti-inflammatory activity of Hydrocortisone 21-acetate was measured using the croton oil-induced ear-swelling assay.²¹ Adult female 1CR mice (20-30 gm; 6 animals/group) were given 10 ul of 25 ug/ul croton oil^{ⓑ1} topically applied to the inner surface of the right ear of each mouse. The croton oil irritant was applied by means of a Hamilton microsyringe^{ⓑ2}. The left ear served as an untreated control (acetone alone produces no effect when applied topically to the ear). The peak swelling occurred six hours later at which time the right (inflamed) and left (control) ears were biopsied using a Baker 6 mm biopsy punch^{ⓑ3}. The ear tissue was weighed to the nearest 0.01 mg on a Mettler balance. The difference in weight between the right and left ears represented the degree of swelling. Each group of animals had its own internal control. Ear weight differences were recorded for each group and the percentage of inhibition of ear swelling was determined.

Hydrocortisone 21-acetate^{ⓑ4}, Aloe vera^{ⓑ5} and combinations were given to treatment groups 30 minutes after the croton oil application. This was necessary to minimize any non-specific interactions between the treatments and the irritant. Hydrocortisone 21-acetate was carefully homogenized into an aqueous solution and topically applied at dosages of 0.1 and 0.5%. Aloe vera was topically applied at dosages of 1.0 and 5.0% .

The systemic influence of Aloe vera on the anti-inflammatory activity of Hydrocortisone 21-acetate was evaluated using a mustard-induced paw-swelling assay. Adult male Sprague-Dawley rats (190 - 260 gm; 12 animals/group) were injected with a 2% mustard solution into the plantar surface of the left hind paw to induce edema. The mustard^{®6} was thoroughly homogenized into a suspension prior to injection. All animals were primed 24 hours prior to edema induction with subcutaneous injections of hydrocortisone 21-acetate at dosages of 0.1 mg/kg or 1.0 mg/kg, Aloe vera at dosages of 25 mg/kg or 100 mg/kg, combinations or saline. Combination injections of hydrocortisone 21-acetate and Aloe vera included : 0.1 mg/kg steroid with 25 mg/kg aloe and 1.0 mg/kg steroid with 100 mg/kg aloe. All injections were given on a 10 ml/kg basis. This regimen was repeated at the time of the injection of 2% mustard. A control group received a plantar saline injection instead of mustard. Animals that received 2% mustard only served as the irritant control group.

Volumetric measurements of the hind paws were taken using a water plethysmometer^{®7.22}. The device consists of a plastic reservoir filled with water in which the left hindpaw of the rat is immersed up to its anatomical hair line. The volume of water displaced by the paw is recorded by the device in units of edema. Readings were taken immediately prior to the plantar injection of 2% mustard or saline and again 6 hours later. The difference between the initial reading and the 6 hour reading was recorded for each group and the percentage of inhibition of edema was determined. Standard curves were developed for both the water and

mercury type plethysmometers. This was done to establish the accuracy, reliability and ease of the newer water type as compared to the already proven mercury instrument.

Polymorphonuclear leukocyte (PMN) infiltration was evaluated by aspirating tissue fluid from the paws. The aspirate was withdrawn using a 27-gauge needle and smeared on albuminized slides. Each slide was stained with Wright's stain¹⁸ and the number of PMNs per high power field was recorded.

Standard errors were determined for each mean value by using the formula: $S.E. = \sqrt{\sum d^2 / N(N-1)}$, where "d" is the deviation of the individual values from the mean and "N-1" represents the degrees of freedom. P values were determined using the students t-test reference.²³

RESULTS AND DISCUSSION

PAW-SWELLING ASSAY (SYSTEMIC)

Anti-inflammatory activity of Aloe vera with hydrocortisone acetate was tested in the paw edema assay. Paw volumes of saline control animals averaged 0.07 ± 0.04 units of edema as compared to the 2% mustard controls which had paw volumes of 0.74 ± 0.07 edema units. This represents a hundred fold increase in paw size over 6 hours resulting from inflammation (Table 1 and Figure 1). The mustard produced an irritant response of 0.81 edema units. Hydrocortisone acetate treated rats receiving the low 0.1 mg/kg steroid dose had an average paw volume of 0.56 ± 0.05 units after 6 hours, a modest 24.9% decrease in edema as compared to mustard controls. The high steroid 1.0 mg/kg dose reduced the paw volume further to 0.49 ± 0.07 units (33.7% edema inhibition). A tendency for a dose-response relationship was recorded but, the values were not significant at the doses used ($P > 0.1$). A paw volume of 0.40 ± 0.06 units was noted by 25 mg/kg dose of decolorized Aloe vera which is a 46.2% decrease in edema. Increasing the Aloe dose to 100 mg/kg reduced the paw volume to 0.34 ± 0.04 units, a 54.2% decrease. Here again, the dose-response was only apparent ($P > 0.1$). However, the comparability of potency between Aloe and hydrocortisone acetate becomes quite evident. The combined treatment of 1.0 mg/kg hydrocortisone acetate and 25 mg/kg Aloe vera produced an average paw volume of 0.25 ± 0.04 units. This represents a 65.7% decrease in edema, a significant ($P < 0.001$) response when compared with the inhibition recorded by Aloe or steroid alone. The higher dose of hydrocortisone acetate (1.0

mg/kg) combined with Aloe vera (100 mg/kg) produced a maximum 88.1% inhibition of edema. This anti-edema response is a maximum response for the assay and is significantly greater than the lower combinational treatment ($P < 0.001$).

Previous work in our laboratory has shown the effectiveness of Aloe vera as an inhibitor of mustard-induced edema in both normal and diabetic rats. In normal animals, a 44.2% decrease in edema was reported.¹⁶ An 80% reduction in mustard-induced edema has been shown in diabetic animals.¹⁴ The systemic activity of Aloe vera has been shown to be effective even against more potent irritants such as carrageenan, kaolin and albumin.¹⁶ The paw edema measurements made in this study were done using the water plethysmometer instead of the mercury plethysmometer which has proved reliable over the past several years in this laboratory. Our conversion to water was an attempt to keep the paw swelling assay "State-of-the-Art". Standard curves developed for both instruments are virtually identical with correlation coefficients of 0.9991 for mercury and 0.9984 for water (Figure 4). This is a nearly perfect correlation for the volume-response relationship. We consider the advantage of the newer water instrument to be its ease and speed of operation. Also, using water as a medium tends to be safer and far more economical than the expensive mercury.

EAR-SWELLING ASSAY (TOPICAL)

The anti-inflammatory activity of Aloe vera given topically on the mouse ear with hydrocortisone acetate against the irritant croton oil is shown in Table 2 and Figure 2. An ear swelling of 6.60 ± 0.74 mg was demonstrated by a topical application of croton

oil in acetone (25 ug/ul). However, no acetone control was needed since it has been established that acetone alone produces no effect when topically applied to the ear. Animals given 1% Aloe vera had an average ear swelling of 5.42 ± 0.74 mg (17.9% inhibition). The higher 5% Aloe vera treated mice recorded an average edema value of 4.70 ± 0.82 (28.8% inhibition). The 0.1% and 0.5% hydrocortisone acetate had ear punch values of 1.77 ± 0.33 and 1.03 ± 0.33 mg which represented edema inhibition values of 73.2% and 84.3%, respectively. One can see the activity relationship between Aloe and hydrocortisone acetate. Aloe vera as a vehicle for the steroid has good anti-inflammatory activity despite the dosage differential. An 85.6% decrease in inflammation was obtained by the combined 1% Aloe plus 0.1% hydrocortisone acetate topical treatment. The edema values can be seen in table 2. Mice receiving 1% Aloe and 0.5% hydrocortisone exhibited a swelling of 0.20 ± 0.29 mg. This represents a 97.0% decrease in croton oil-induced edema. The presence of the Aloe vera has significantly added to the biological activity of the steroid ($P < 0.001$). This means that the dosage of steroid could be reduced and the biological activity could be maintained by a natural substance suggesting that Aloe vera is a valuable biological vehicle for the corticosteroid. Thus, Aloe vera provides the podiatrist with a valuable tool in using steroids against inflammation. In previous reports using Aloe vera alone, at 1% topical applications, we have been able to inhibit inflammation by as much as 67.4%.²⁴

The paw-swelling assay (a biological standard for anti-inflammation) allows us to evaluate the anti-inflammatory systemic activity of Aloe vera alone, and in combination with hydrocortisone acetate. This assay is important because it rules out the possibility of an antagonistic interaction which would preclude the use of Aloe vera as a vehicle for the steroid. It provides a good predictive index for clinical success with a high degree of precision and low experimental error. The data from paw swelling shows that Aloe vera at a dosage of 25 mg/kg has anti-inflammatory activity exceeding that of hydrocortisone at dosages of 0.1 and 1.0 mg/kg. The activity of 100 mg/kg Aloe vera exceeded the activity of 25 mg/kg Aloe vera by only 8%, indicating that the higher dose is approaching the maximum effective dose on the dose-response curve. Thus, at the doses used, a dose-response relationship can only be apparent. The combined treatment groups of Aloe vera and hydrocortisone showed an additive response against the mustard-induced edema. This strongly supports the use of Aloe vera as a biologically active vehicle. A biological vehicle acts as a carrier for the steroid (so that the steroid can inhibit the inflammation) yet at the same time contributes to the biological activity. The vehicle contribution made by Aloe may be great in view of the fact that so little steroid is absorbed.

The topical ear-swelling assay also showed an additive effect of Aloe vera with the steroid. This assay has important significance since the most common use for corticosteroids is topical. Aloe vera contributes to the overall anti-inflammatory effect in two ways. Firstly, by its own anti-inflammatory

activity acting in concert with hydrocortisone and secondly, it hydrates the stratum corneum allowing increased absorption of the steroid. Again, this means less steroid is used without a compromise in activity and, at the same time, the risk of dose related side effects is diminished. Traditionally, physicians have been weary of the steroid side effects. Aloe vera may provide a means of reducing the steroid dose to reduce or eliminate the risk of side effects in both acute and chronic inflammation.

PMN INFILTRATION

The movement of polymorphonuclear leukocytes into the site of inflammation provides us with a most sensitive and accurate model for evaluating anti-inflammatory agents.¹⁸ Fluid aspirated from the inflamed paws was used to evaluate the inhibitory activity of Aloe vera with hydrocortisone acetate on polymorphonuclear leukocyte infiltration (Table 3 and Figure 3). Saline control paws had an average PMN count of 1.2 ± 0.22 per high power field (HPF). The PMN count in the aspirate from 2% mustard control paws increased to an average of 3.8 ± 0.33 . The mustard produced a 3.2 fold increase in PMN migration into the inflammation site. A PMN count of 1.3 ± 0.12 was recorded for the animals receiving 0.1 mg/kg hydrocortisone acetate, representing a 66.4% reduction as compared to the mustard control. Hydrocortisone acetate (1.0 mg/kg) resulted in a count of 0.9 ± 0.09 , a 75.4% reduction. The low 25 mg/kg Aloe vera produced a PMN migration of 1.4 ± 0.09 (64.3% reduction). Increasing the Aloe vera dose to 100 mg/kg reduced the count to

1.0±0.09, a 73.5% inhibition. The combined treatment of Aloe vera (25 mg/kg) and hydrocortisone acetate (0.1 mg/kg) showed an 84.0% inhibition. The higher dose combination of Aloe vera and hydrocortisone produced a 91.1% reduction, at respective doses of 100 mg/kg and 1.0 mg/kg. These results agree well with the paw and ear swelling data. It also seems to indicate that Aloe works at a cellular level since PMNs have a central role in inflammation. Previous studies in this lab have shown decolorized Aloe vera to inhibit PMN migration into the inflamed paw 40.6%.¹²

Aloe vera at 25 mg/kg was 97% as effective in reducing PMN infiltration as hydrocortisone given at 0.1 mg/kg. Similarly, 100 mg/kg Aloe vera had a comparable (97%) effectiveness to hydrocortisone at 1.0 mg/kg. This demonstrates that at a comparable dose, Aloe vera is equally effective as hydrocortisone in inhibiting the inflammatory response at the cellular level. The combined treatment groups, while not purely additive, showed an additive trend. Since PMNs play a major role in the inflammatory process, these findings are important because a good anti-inflammatory agent must be effective at the tissue and cellular levels. It is tempting to estimate what contribution Aloe makes toward the steroid anti-inflammatory activity in these assays.

PMNs are part of the body's first line of defense against bacterial invasion. By a process called diapedesis, PMNs "squeeze" between the endothelial cells of postcapillary venuoles into the surrounding connective tissue where they are active

(figure 5). The action of PMNs in inflammation has been described as a "two-edged sword". On one hand, they are able to phagocytize offending agents such as bacteria and particulate matter and, at the same time, cause further tissue damage. One reason for this is that during phagocytosis, or upon death of the PMN, destructive lysosomal enzymes are released. These enzymes cause tissue damage to the host which leads to an immunological reaction.²⁵ Circulating antibodies are bound to mast cells which are lysed upon contact and reaction with the antigen. Several vasoactive substances such as histamine, prostaglandins and kinins are then released from the mast cell. The deposition of the insoluble immune complexes can cause tissue damage directly and also by activating the lysosomal system of PMNs.²⁵ Since Aloe vera reduces PMN infiltration and pain, the many points of influence on inflammation can be estimated as well as the possible multifold approach the podiatrist has for treating patients. Inflammation is a homeostatic protective mechanism for ridding the body of a noxious agent or a way of removing a primary damage caused by a deleterious influence. After the inflammation the process of tissue repair ensues. Inflammation and tissue repair are interwoven processes in response to injury. Glucocorticoids are good anti-inflammatory agents but are poor wound healers and repair agents. Steroids tend to reduce connective tissue and prevent wound healing.²⁶ Aloe vera, on the other hand, can inhibit inflammation and increase the repair process since it improves wound healing.²⁷ The combination of Aloe vera with steroids in the treatment of inflammation has broad implications as a tool to be used by physicians.

OXYGEN RADICALS AND MEDIATOR PRODUCTION

Oxygen radicals, produced by PMNs, damage tissue and produce inflammation. PMNs also produce mediators that work with oxidants to establish inflammation and encourage the recruitment of inflammatory cells (Figure 6). Aloe vera and steroids influence oxygen radical and mediator production. When challenged to phagocytosis, the PMN exhibits a burst of oxygen consumption. Oxidases on the PMN plasma membrane are responsible for this respiratory burst during which glucose metabolism increases 10 fold via the hexose monophosphate pathway. During this period of increased oxygen use, the PMN produces several oxygen radicals which have bactericidal activity.²⁸ Oxygen radicals are highly reactive, and short lived species generated by PMNs during phagocytic activity in sites of acute inflammation. These species include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen (1O_2), and hydroxy radicals (OH^-).²⁹ While free radicals have beneficial antibacterial activity, they also cause further insult to vascular endothelium and other tissues. Oxygen radicals have been implicated in phospholipid membrane damage, connective tissue and synovial fluid degradation, increased vascular permeability and cell death.²⁹ The influence of steroids and Aloe vera on inflammation and arthritis must be explained in the light of what we know about oxygen radicals produced by phagocytic cells.

Biological membranes are rich in polyunsaturated lipid which makes them vulnerable targets for oxygen radical attack. Oxygen radicals initiate a cascade of reactions involving

arachidonic acid, ultimately leading to the production of mediators such as prostaglandins, thromboxanes and leukotrienes.³⁰ In this process more oxygen radicals are produced. Oxygen radicals act as a stimulus which activates the enzyme phospholipase, which cleaves arachidonic acid from cell membranes.²⁹ This free arachidonic acid may enter two metabolic pathways as depicted in figure 7. The dominant cyclooxygenase pathway results in prostaglandin and thromboxane production, while the lipoxygenase pathway results in leukotriene formation. The products of the cyclooxygenase pathway, prostaglandins, cause vasodilation and at the same time, stimulate other mediators such as histamine, serotonin, kinins and complement.³¹ These mediators cause an increase in vascular permeability which leads to PMN aggregation and increased oxygen radical production.³¹ The free radicals produced in the cyclooxygenase pathway are believed to act as feedback inhibitors, thus regulating prostaglandin synthesis.²⁹ Leukotrienes are formed via the lipoxygenase pathway. These are chemotactic agents that cause the aggregation of PMNs and macrophages into the area of insult.³² The PMNs increase the concentration of free radicals which can lead to further tissue damage.

Cells are constantly exposed to toxic oxygen-derived products as a consequence of their normal oxidative metabolism. The superoxide dismutases, peroxidases and catalases function as an endogenous defensive team which tries to reduce the exposure of cells to free radicals.³³ Superoxide radicals exert their deleterious effects directly on cells as well as by dismutating

to form hydrogen peroxide which is a strong oxidant in its own right. The primary defense against superoxides are the metalloenzymes called superoxide dismutases which catalyze the conversion of O_2^- to H_2O_2 which can then be reduced to yield the highly reactive hydroxyl radical.³³ A number of endogenous defense mechanisms exist to prevent the accumulation of hydrogen peroxide. Peroxidases and catalases are the primary enzymes that perform this function. Catalases dismute H_2O_2 into H_2O and O_2 while the peroxidases catalyze the reduction of H_2O_2 .³³ During inflammation these protective mechanisms can be overwhelmed. Superoxide dismutases have been used therapeutically to reduce inflammation, radiation damage, tumor promotion and hyperoxia.²⁹

A basic understanding of the biochemistry of inflammation is critical for the development of a successful therapy regimen. Anti-inflammatory agents can exert their effect at a variety of locations in the biological scheme of inflammation. Steroids are non-specific agents and the most well understood. Drugs such as hydrocortisone, act by blocking the enzyme phospholipase A_2 thus preventing the formation of arachidonic acid.³⁴ By blocking prostaglandins, steroids prevent the release of vasoactive amines, such as histamine and serotonin, and the formation of the chemotactic leukotrienes. While steroids are effective in blocking these components of inflammation, the already discussed toxicity remains as a significant problem.

The newer non-steroidal anti-inflammatory drugs (NSAIDs) have their action not on phospholipase but, are specific for the cyclooxygenase pathway.³⁵ This activity also allows NSAIDs to block prostaglandin synthesis like steroids. The problem with

most of these drugs is that they do not effect the lipooxygenase pathway, thus leukotrienes are still produced and PMN infiltration may yet pose a problem in certain inflammatory situations (figure 7).³² Using these data as a background, one can attempt to speculate on "how Aloe vera works" so as to maximize Aloe vera's clinical potential in the management of inflammation.

MECHANISMS OF ALOE VERA ACTIVITY

The complexity of Aloe vera's components makes the study of its anti-inflammatory activity a difficult task. Yet, several points can be discussed with regard to mechanisms. Aloe vera does not have a single mechanism of action. Aloe vera contains amino acids such as phenylalanine and tryptophane, which have anti-inflammatory activity.¹¹ The salicylic acid in Aloe prevents the biosynthesis of prostaglandins from arachidonic acid. This explains, in part, how Aloe reduces vasodilation and decreases the vascular effects of histamine, serotonin and other mediators of inflammation. Since prostaglandins play an integral role in regulating both inflammatory and immune reactions, Aloe vera can effect both of these systems by blocking prostaglandin synthesis. Aloe also contains bradykinase which breaks down bradykinin, a vasodilator, and at the same time, stimulates angiotensin, a potent vasoconstrictor.³⁶ Both stimulatory and inhibitory components have been identified in Aloe.³⁷ This means that Aloe can act as a modulator of both immune and inflammatory reactions. Aloe vera has been shown to have an immuno-suppressive action in adjuvant-induced arthritis.³⁸ On the other hand, Aloe can also

act as a stimulator of wound healing and antibody production.³⁹ As well as acting as a prostaglandin blocker, Aloe acts as an immune-modulator by influencing the production of lymphocyte- and macrophage-derived mediators (lymphokinins) including migration inhibitory factor, interleukins and interferon.³²

Besides Aloe's effects on inflammatory and immune reactions, it is likely that it also acts as a scavenger of free oxygen radicals. Vitamin C in Aloe has both a topical and systemic influence against the inflammation seen in adjuvant arthritis.⁴⁰ Oxygen radicals are probably "picked up" or scavenged by vitamin C to reduce inflammation.²⁹ It has long been recognized that vitamin E, a known anti-oxidant, is a component of Aloe vera.¹¹ Catalase has also been identified in Aloe which, as already described, catalyzes the conversion of H_2O_2 to H_2O and O_2 .¹¹

ALOE AS A VEHICLE

Since Aloe vera contains 99.5% water and 0.5% solids, water tends to move from the surface of the skin through the stratum corneum barrier to the vascular dermal layer. Aloe contains many water soluble compounds such as enzymes, amino acids and carbohydrates as well as oil soluble compounds such as vitamins, sterols and anthraquinones.¹¹ The water soluble characteristics of Aloe seem to originate from the gel of the leaf while the ability to solubilize oil and hydrophobic compounds probably comes from the outer rind. We feel that pharmacologic agents of both solubilities can be placed in Aloe and carried through the epidermal barrier to blood vessels in the dermis. Studies of vitamin C activity with Aloe vera (topical and systemic) also

support the effectiveness of Aloe vera as a vehicle for water soluble compounds.⁴⁰ In the present work, Aloe vera allowed the steroid to maintain its activity when given subcutaneously or topically at low doses. Furthermore, the compounds present in Aloe's 0.5% solid component contributed in an additive way to the activity of hydrocortisone acetate. If the dose-response curve was extended out, one might encounter a synergism (no evidence of a synergism was recorded). The additive influence of Aloe leads us to believe that Aloe vera is a good vehicle for steroids.

Aloe vera hydrates and softens the skin as oils do. Yet, it also covers the skin in an occlusive manner to prevent the loss of water which is necessary for wound healing. Aloe permeability through the skin makes it a unique vehicle that would allow the incorporation of both water and oil soluble agents. The stratum corneum acts as a barrier to steroid penetration.⁴¹ Since Aloe vera hydrates the stratum corneum, this barrier is rendered less effective thus, steroid penetration of the keratin layer is increased. Aloe's large amounts of fluid can provide a way for both hydrophilic and hydrophobic compounds to move across the skin. Hydrophobic compounds can negotiate the lipid aspects of this barrier by means of Aloe vera as well. Aloe vera fluid can go around and through cells to overcome the skin barrier carrying oil and water soluble compounds.⁴² Keratin and lipid resistance of the skin is reduced by this "dual property" of Aloe. An agent like the steroid can be effective if the skin-aloe interface has maximum hydration, if the Aloe has a low affinity for the steroid and, if the vehicle contact time with the skin is adequate. These criteria are met by Aloe vera.

SUMMARY

Podiatrists struggle in treating inflammation. They have become well aware of the pitfalls of using steroids. With this in mind, our study focused on Aloe vera as a vehicle for hydrocortisone acetate. Aloe's novel in vivo activity complements the steroid while reducing its toxic effects. The assays used successfully demonstrated the anti-inflammatory activity of Aloe vera alone and in combination with hydrocortisone acetate. The Aloe worked well with the steroid when administered systemically in the paw-swelling assay (88.1% edema reduction). The ear-swelling assay enables us to look at Aloe as it might act when incorporated into a steroidal preparation as a vehicle. This assay showed a classic additive response (97% reduction of edema). Together, the two animal models allowed us to rule out the possibility of an antagonism that would disqualify Aloe as a vehicle for steroids. In combination, Aloe and hydrocortisone maintained their effectiveness at the cellular level as represented by the 91.1% reduction in polymorphonuclear leukocytes. An attempt was made to explain "how Aloe vera works" in terms of the various mediators of inflammation and in relation to oxygen radicals.

Future studies of Aloe vera will explore the biological activity of various extracts of the gel in hope of identifying the active agent(s). An attempt will be made to quantify the effects of Aloe vera and its extracts on oxygen radical production as well. This study does not suggest the use of Aloe

vera as a replacement, but rather an aid for current steroid therapies. It has been our purpose to increase the effectiveness of this type of therapy in the management of inflammation using a natural substance. We continue to research Aloe vera in hope of providing podiatric physicians with safe and effective alternatives to manage diseases of the lower extremity.

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TRADE NAMES CITED

- Ⓡ¹ Sigma Chemical Co., St. Louis, Missouri.
- Ⓡ² Hamilton Co., Reno, Nevada.
- Ⓡ³ Key Pharmaceuticals, Miami, Florida.
- Ⓡ⁴ Sigma Chemical Co., St. Louis, Missouri.
- Ⓡ⁵ Florida Food Products Inc., Eustis, Florida.
- Ⓡ⁶ McCormick and Co. Inc., Hunt Valley, Maryland.
- Ⓡ⁷ Ugo, Basile, Milan, Italy.
- Ⓡ⁸ Harleco, EM Science, Gibbstown, New Jersey.

Figure 1. Anti-Inflammatory Activity of Aloe Vera given
Subcutaneously as a Vehicle for Hydrocortisone
Acetate.

ANTI-INFLAMMATORY ACTIVITY OF ALOE VERA GIVEN SUBCUTANEOUSLY AS A VEHICLE FOR HYDROCORTISONE ACETATE

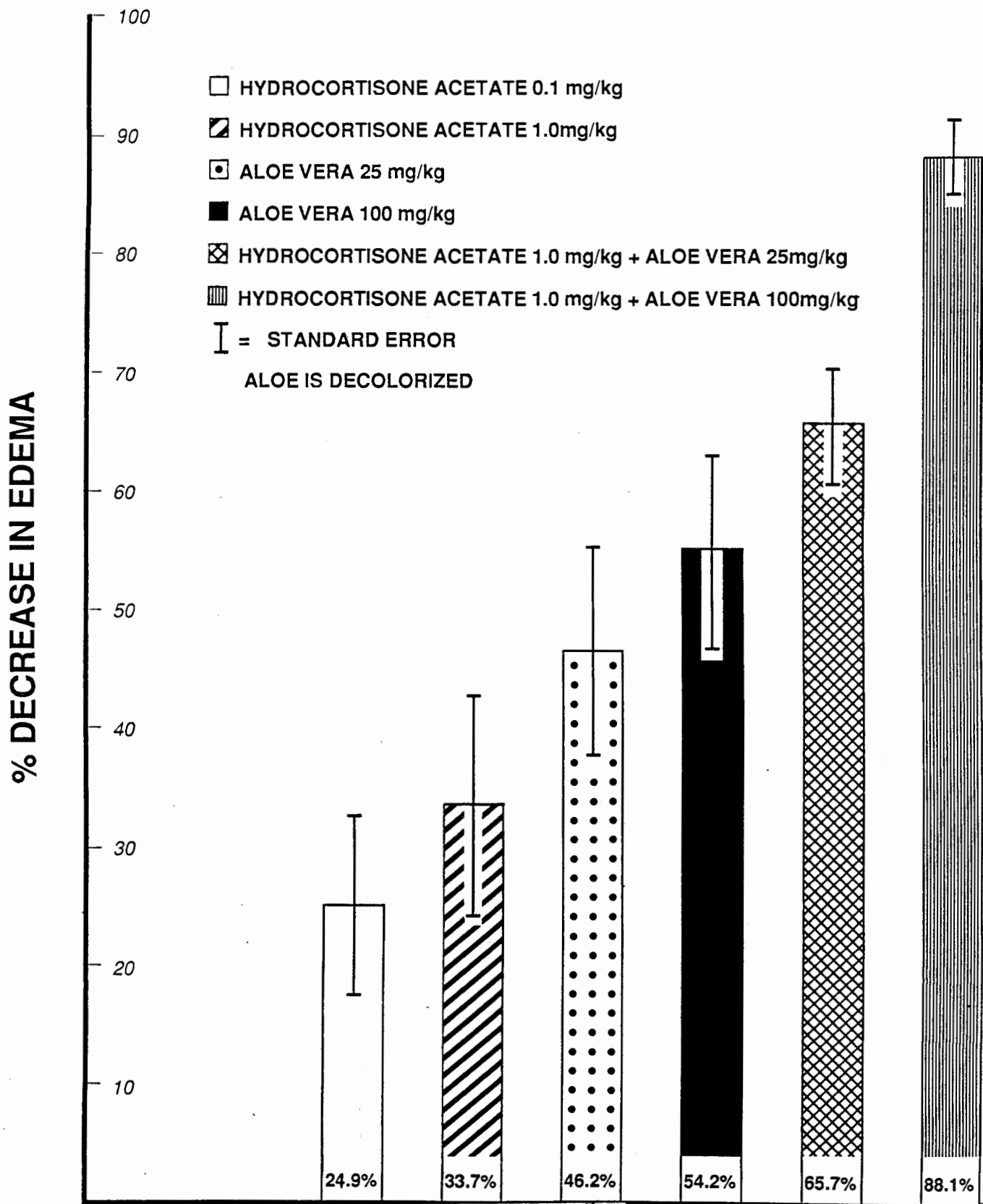


FIGURE 1

Figure 2. Anti-Inflammatory Activity of Aloe Vera given
Topically as a Vehicle for Hydrocortisone Acetate.

ANTI-INFLAMMATORY ACTIVITY OF ALOE VERA GIVEN TOPICALLY AS A VEHICLE FOR HYDROCORTISONE ACETATE

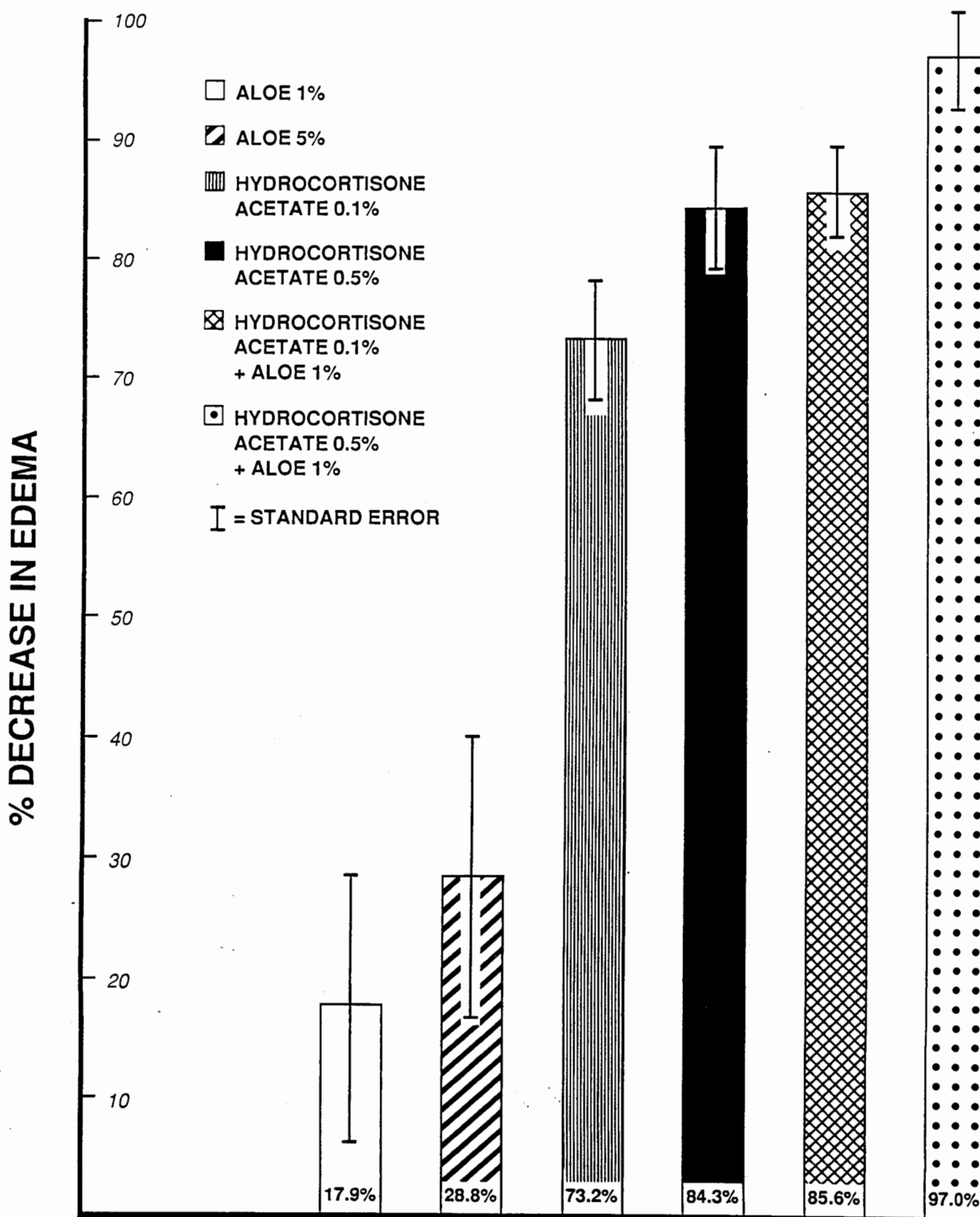


FIGURE 2

Figure 3. The Effect of Aloe Vera and Hydrocortisone on PMN
Infiltration into a Mustard Induced Inflammation
Site in Rats.

THE EFFECT OF ALOE VERA AND HYDROCORTISONE ON PMN INFILTRATION INTO A MUSTARD INDUCED INFLAMMATION SITE IN RATS

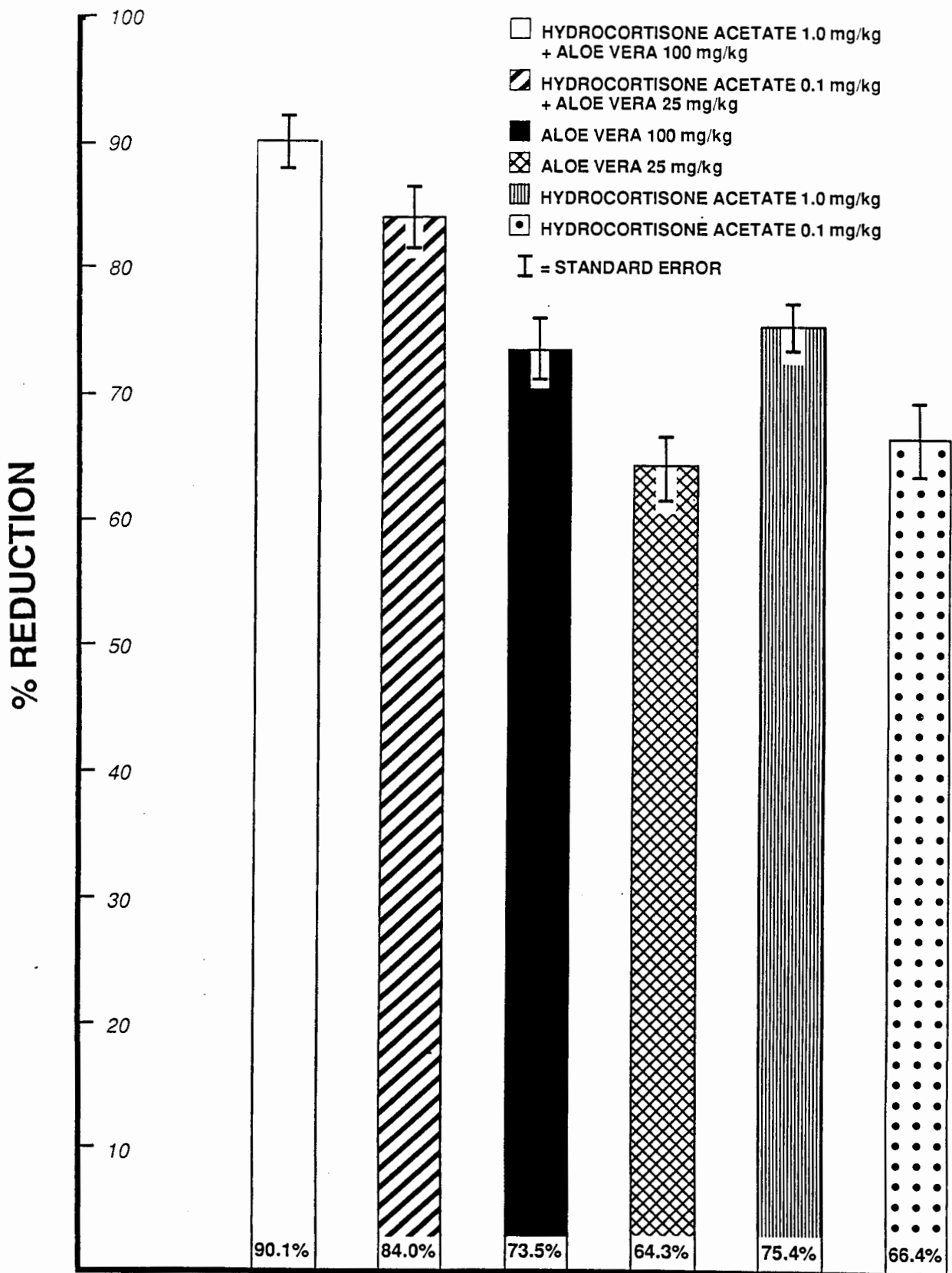


FIGURE 3

Figure 4. A Comparison of Water and Mercury Plethysmography.

A COMPARISON OF WATER AND MERCURY PLETHYSMOGRAPHY

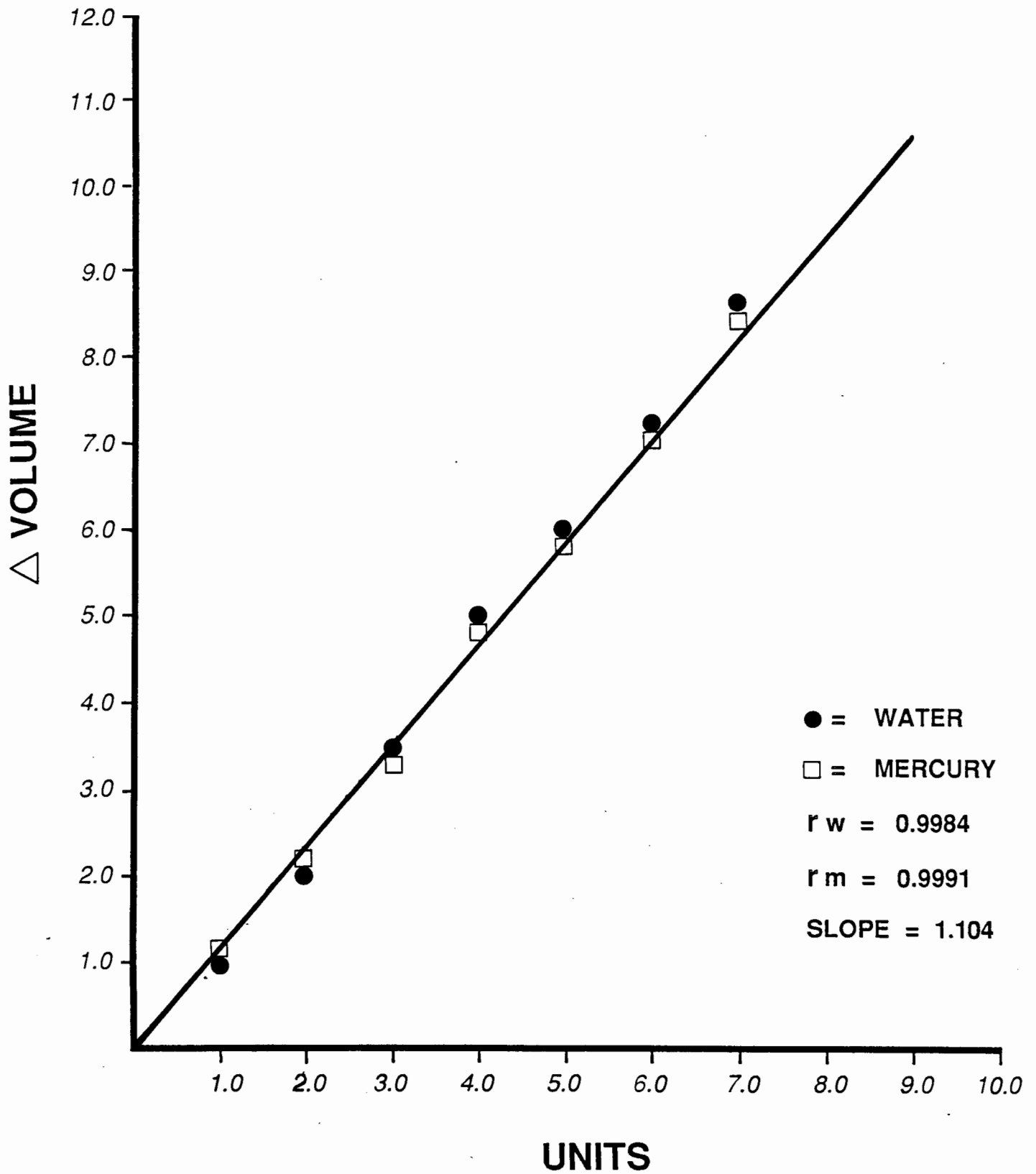


FIGURE 4

Figure 5. Acute Inflammatory Response.

ACUTE INFLAMMATORY RESPONSE

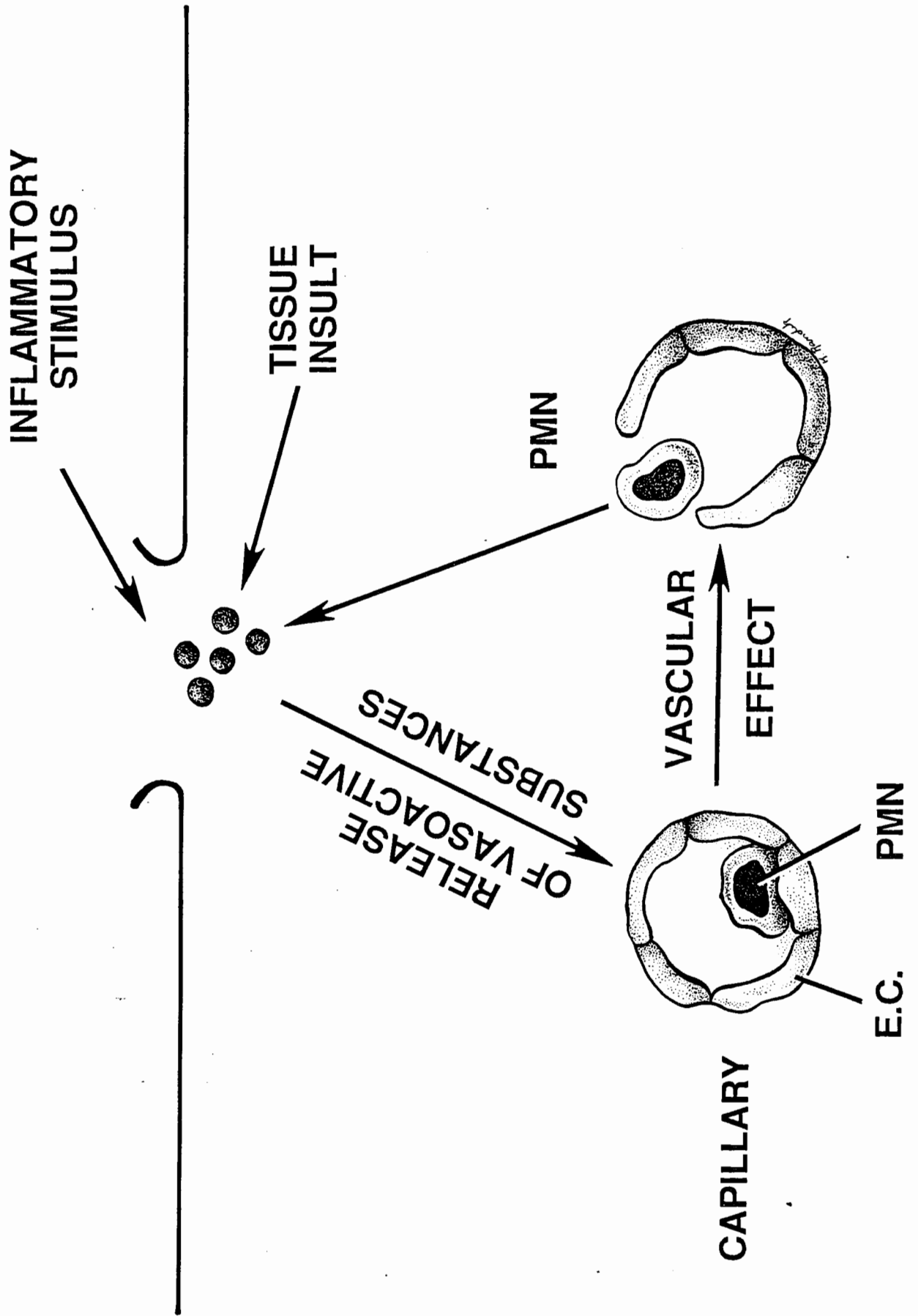


FIGURE 5

Figure 6. Oxygen Radicals in Inflammation.

OXYGEN RADICALS IN INFLAMMATION

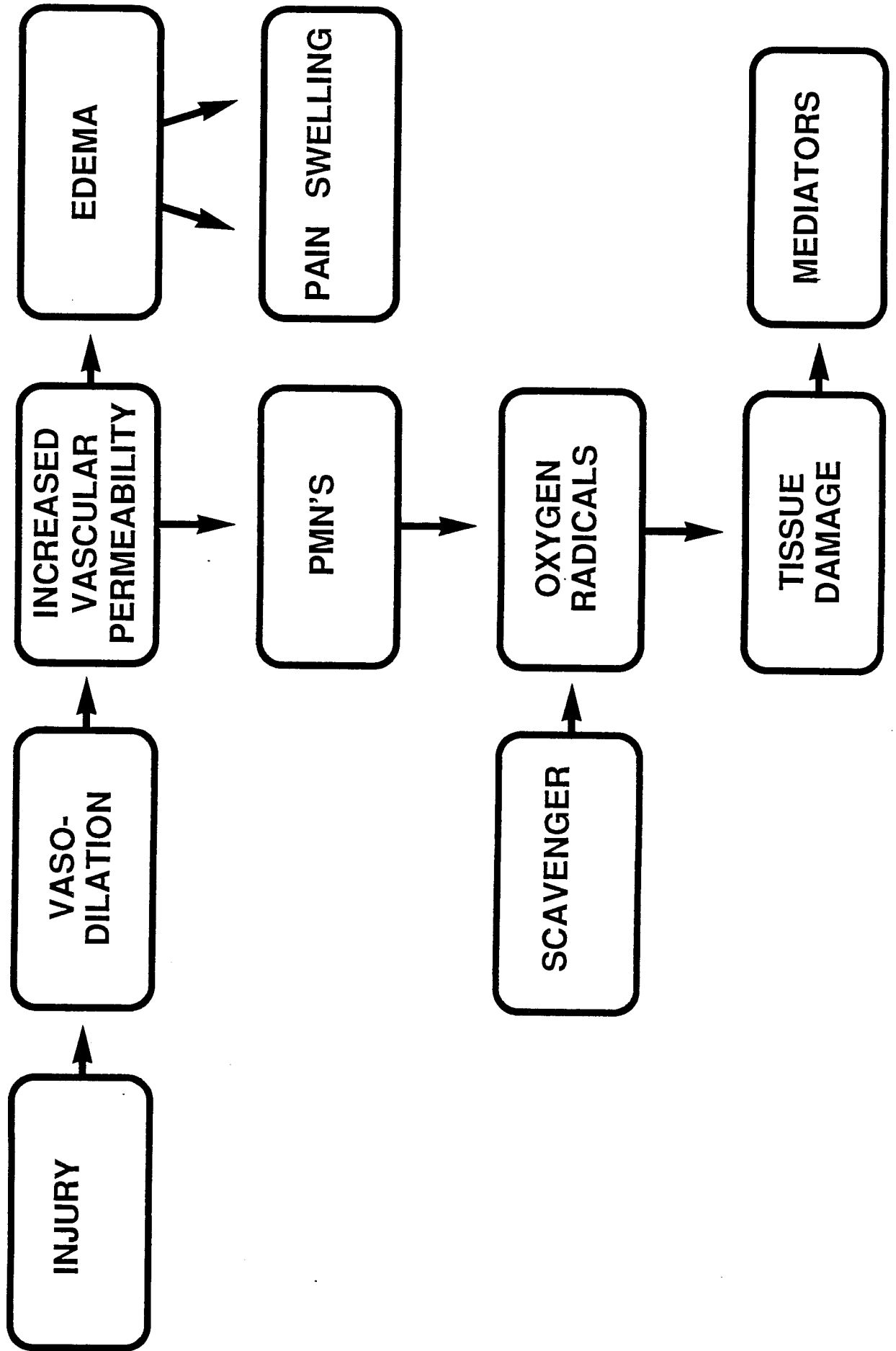


FIGURE 6

Figure 7. Arachidonic Acid Metabolism and the Sites of Anti-inflammatory Drug Activity.

ARACHADONIC ACID METABOLISM AND THE SITES OF ANTI-INFLAMMATORY DRUG ACTIVITY

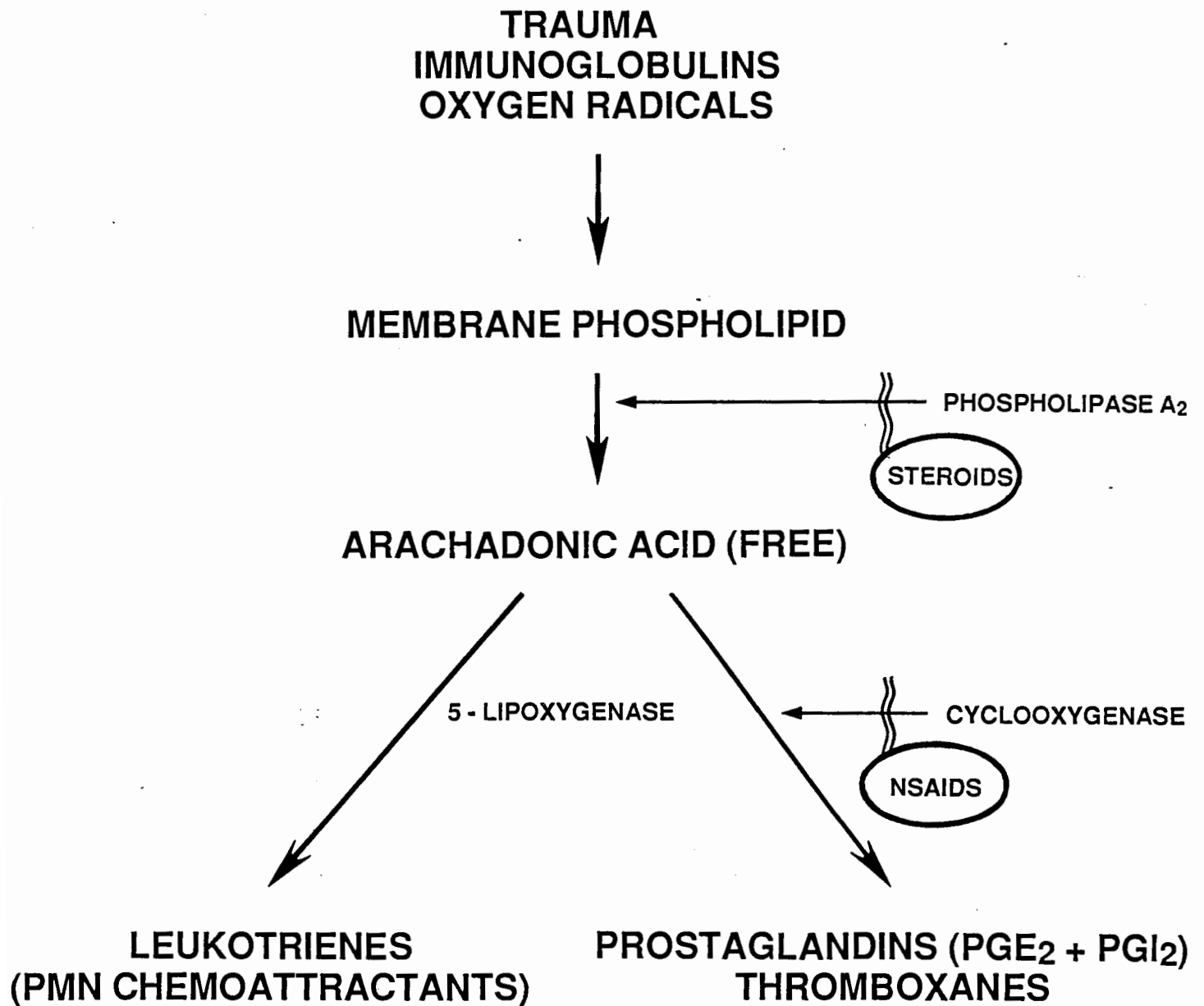


FIGURE 7

Table 1. Anti-Inflammatory Activity of Aloe Vera given
Subcutaneously as a Vehicle for Hydrocortisone
Acetate.

ANTI-INFLAMMATORY ACTIVITY OF ALOE VERA GIVEN SUBCUTANEOUSLY AS A VEHICLE FOR HYDROCORTISONE ACETATE

<u>TREATMENT</u>	<u>EDEMA</u>	
	<u>UNIT VOLUME</u>	<u>% DECREASE</u>
SALINE CONTROL	- 0.07 ± 0.04	—
2% MUSTARD	0.74 ± 0.07	—
+ HYDROCORTISONE ACETATE 0.1 mg/kg	0.56 ± 0.05	24.9 ± 7.4
+ HYDROCORTISONE ACETATE 1.0 mg/kg	0.49 ± 0.07	33.7 ± 8.9
+ ALOE VERA 25 mg/kg	0.40 ± 0.06	46.2 ± 8.3
+ ALOE VERA 100 mg/kg	0.34 ± 0.04	54.2 ± 4.5
+ HYDROCORTISONE ACETATE 1.0 mg/kg + ALOE VERA 25 mg/kg	0.25 ± 0.04	65.7 ± 5.5
+ HYDROCORTISONE ACETATE 1.0 mg/kg + ALOE VERA 100mg/kg	0.09 ± 0.02	88.1 ± 3.1

± = Standard error; 12 Rats / group; Aloe vera is decolorized.

Table 2. Anti-Inflammatory Activity of Aloe Vera given
Topically as a Vehicle for Hydrocortisone Acetate.

ANTI-INFLAMMATORY ACTIVITY OF ALOE VERA GIVEN TOPICALLY AS A VEHICLE FOR HYDROCORTISONE ACETATE

<u>TREATMENT</u>	<u>EDEMA</u>	
	<u>EAR TISSUE WEIGHT CHANGE (mg)</u>	<u>% DECREASE</u>
CROTON OIL CONTROL 25 µg/µl	6.60 ± 0.74	—
+ ALOE VERA 1%	5.42 ± 0.74	17.9 ± 10.9
+ ALOE VERA 5%	4.70 ± 0.82	28.8 ± 12.2
+ HYDROCORTISONE ACETATE 0.1%	1.77 ± 0.33	73.2 ± 4.9
+ HYDROCORTISONE ACETATE 0.5%	1.03 ± 0.33	84.3 ± 5.1
+ HYDROCORTISONE ACETATE 0.1% + ALOE VERA 1%	0.95 ± 0.25	85.6 ± 3.4
+ HYDROCORTISONE ACETATE 0.5% + ALOE VERA 1%	0.20 ± 0.29	97.0 ± 4.2

± = Standard error; Aloe vera is decolorized

Table 3. The Effect of Aloe Vera and Hydrocortisone on PMN
Infiltration into a Mustard Induced Inflammation
Site in Rats.

THE EFFECT OF ALOE VERA AND HYDROCORTISONE ON PMN INFILTRATION INTO A MUSTARD INDUCED INFLAMMATION SITE IN RATS

<u>TREATMENT</u>	<u>PMN COUNT No. / HPF</u>	<u>% REDUCTION</u>
SALINE CONTROL	1.2 ± 0.22	—————
2% MUSTARD CONTROL	3.8 ± 0.33	—————
+ HYDROCORTISONE ACETATE 0.1mg / kg	1.3 ± 0.12	66.4 ± 2.9
+ HYDROCORTISONE ACETATE 1.0mg / kg	0.9 ± 0.09	75.4 ± 2.0
+ ALOE VERA 25mg / kg	1.4 ± 0.09	64.3 ± 2.5
+ ALOE VERA 100 mg / kg	1.0 ± 0.09	73.5 ± 2.7
+ HYDROCORTISONE ACETATE 0.1 mg / kg	0.6 ± 0.12	84.0 ± 2.7
+ ALOE VERA 25mg / kg		
+ HYDROCORTISONE ACETATE 1.0mg / kg	0.4 ± 0.09	90.1 ± 2.1
+ ALOE VERA 100 mg / kg		

± = Standard error; 12 Rats / group; Aloe vera is decolorized