Multiple Topical Applications of Arachidonic Acid to Mouse Ears Induce Inflammatory and Proliferative Changes

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The response to daily topical applications of arachidonic acid (0.25 − 4 mg/ear/day) to the ears of outbred CD-1 mice was monitored. The first application produced erythema, extravasation of plasma proteins resulting in an increase in ear weight, and some neutrophil accumulation (detected histologically and quantified by myeloperoxidase content). The second application produced minimal edema but did cause erythema and a greater accumulation of neutrophils. Subsequent daily application caused erythema, neutrophil accumulation, and an increase in ear weight predominantly due to cell proliferation (epidermis and connective tissue). Daily applications of other unsaturated fatty acids did not match the response induced by arachidonic acid. Mast cell deficient mice (W/W^v) exhibited a smaller edema response to the first dose of arachidonic acid compared to either their wild-type controls or CD-1 mice. In addition, W/W^v mice exhibited a smaller ear weight increase and myeloperoxidase accumulation following eight daily doses of arachidonic acid. However, epidermal proliferation was similar in all the strains of mice tested. These data suggest that the edema caused by the first topical application of arachidonic acid is partly mast cell mediated. Mast cells also appear to be involved in the neutrophil infiltration induced by multiple topical applications, but not in the epidermal proliferation. *J Invest Dermatol 91:298–302, 1988*

Arachidonic acid and its metabolites have been implicated in the pathogenesis of a number of inflammatory skin diseases [1–3]. This has created a need for animal models that would permit further analysis of the pathologic roles of the eicosanoids. Recent reports have demonstrated that a single topical application of high doses of arachidonic acid to mouse ears induces the rapid onset of erythema, edema, and neutrophil accumulation [4–7]. Products of arachidonic acid metabolism have been implicated as mediators of these responses [4–7], and increased levels of arachidonic acid metabolites have been found in the arachidonic acid-treated ears [6]. These observations have resulted in the use of this model for the evaluation of novel therapeutic agents [8,10–12], despite the abundant pharmacologic evidence for the involvement of other mediators [8–12]. Other studies have shown that multiple topical applications of arachidonic acid to guinea pig [13] or mouse [14] ears induced marked keratinocyte proliferation. Therefore, study of the response to topical application of arachidonic acid, because it induces a number of the important inflammatory and proliferative features of chronic inflammatory skin diseases, could increase understanding of the potential pathologic role of eicosanoids in skin. The present report compares the response to a single topical application of arachidonic acid with the response to multiple daily topical applications, in order to lay the groundwork for future pharmacologic studies. A significant contribution from connective tissue mast cells to the edema response, but not the proliferative response, has been identified.

METHODS

Male, random-bred CD-1 mice were obtained from Charles River Breeding Laboratories (Wilmington, MA). Male $WBB6F_1/J\text{-}\underline{W}/\underline{W}^v$ mast-cell deficient mice (W/W^v) and their wild-type controls, $WBB6F_1/J\text{-}\underline{W}/+$ and $WBB6JF_1/J\text{-}\underline{W}^v/+$ ($W/+$), were obtained from Jackson Laboratories (Bar Harbor, Maine). Mice were fed standard laboratory diet and tap water ad lib and were used at 25–35 g body weight.

Fatty acids were obtained from Nu-Chek Prep (Elisian, MN), dissolved in reagent grade acetone, and stored at −70°C until used. The arachidonic acid used was greater than 99% pure and contained no detectable hydroxy derivatives (<.1%). The major impurity was a single spot on thin layer chromatography which had an R_f consistent with eicosatrienoic acid (manufacturers information). The fatty acid solutions, or vehicle (20 μl), were applied to the dorsal surface of the pinna of the right ear. Care was taken to distribute the agent evenly and to minimize spreading of the solution onto the skull. The mice were examined daily and macroscopic changes (erythema, crusting, discoloration) in the ears noted. After the appropriate number of daily applications, the mice were killed by cervical dislocation 1 h after the last treatment, unless otherwise indicated. Discs of 9-mm diameter were cut with a cork borer from both the right (treated) and left (untreated) ears, weighed immediately, and the percent increase in the weight of the treated compared to the untreated ear was calculated. Preliminary studies indicated that there was no significant difference in the weights of discs from left and right ears of untreated mice (range 15–22 mg).

For histologic evaluation, the discs from treated ears were fixed in 10% neutral buffered formalin, embedded in paraffin, and 5 μ sections cut along the midline running from base to tip. Sections were

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stained with hematoxylin and eosin or toluidine blue. The thickness of the dorsal epidermis, from the basal layer to the stratum granulosum, was measured at five randomly chosen locations, with an ocular micrometer. The mean of the five values was used for statistical analysis.

The myeloperoxidase content of the ear discs was determined in order to assess neutrophil (PMN) infiltration [6,15,16]. Discs were homogenized (using a Polytron PT10 homogenizer at a power setting of 10 for 15 sec) in 10 ml of ice-cold phosphate buffer (pH 6.0, 50 mM) containing hexadecyltrimethylammonium bromide (0.5% w/v; Sigma Chemical Co., St. Louis, MO). The homogenates were then centrifuged (100 × g for 10 min), the supernatants filtered through Whatman No. 1 paper, and the myeloperoxidase activity measured using a kinetic assay with o-diansidine dihydrochloride as substrate [16]. In order to ensure that myeloperoxidase activity showed a linear relationship with neutrophil numbers, the assay was calibrated with a homogenate of a known number of rat, casein-induced peritoneal neutrophils. The myeloperoxidase activity of the discs is expressed as the equivalent number of rat neutrophils. These numbers may not accurately reflect the absolute number of mouse neutrophils present, but they enable valid comparisons to be made between groups within an experiment.

In order to measure plasma extravasation, the mice were injected i.v. with 10 ml/kg of a 1% solution of Evans Blue (MCB, Norwood, OH) immediately before application of arachidonic acid. One hour after application of arachidonic acid, the mice were killed and ear discs cut as described above. The ear discs were then digested in 1 ml of NCS tissue solubilizer (Amersham Corp., Arlington Heights, IL) plus 0.2 ml water at 37°C overnight. The Evans Blue was extracted from the digest and measured spectrophotometrically at 620 nm as described by others [5]. The results are reported as the absorbance of the arachidonic acid-treated ear minus that of the untreated ear [5].

Data were analyzed by one-way analysis of variance followed by Dunnett's test for multiple comparisons with a control group.

RESULTS

Initial studies confirmed previous reports [4,5,7] that a single topical application of a large dose of arachidonic acid (4 mg) to mouse ears caused an increase in ear weight which reached a plateau in 1 h, then slowly declined (data not shown). Unless otherwise indicated, the response was measured 1 h after the last application of arachidonic acid in all subsequent studies. The increase in ear weight in response to a single application of arachidonic acid was dose-related over the range of doses studied (Day 1 in Fig 1). As has been reported by others, a second application of arachidonic acid, 24 h after the first, produced a much smaller increase in ear weight compared to the first application (Day 2 in Fig 1). However, further daily applications resulted in a gradual increase in ear weight which reached a plateau after 5 d. Multiple doses of 4 mg/ear produced the largest increase in ear weight. The responses to multiple applications of 1 and 0.25 mg/ear were not significantly different from each other (analysis of variance and Dunnets multiple comparison test), although they were significantly greater than the response to the vehicle (acetone) (Fig 1).

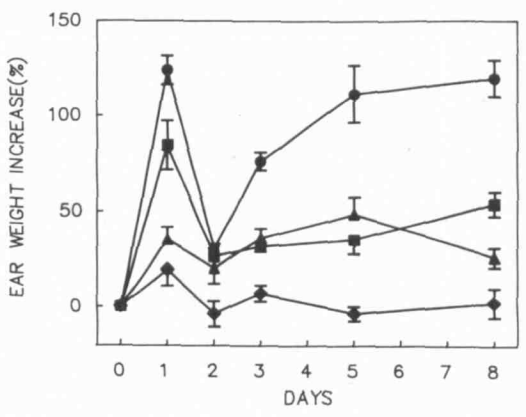

Figure 1. Topical application of arachidonic acid induces increased ear weight. Groups of five mice were killed 1 h after the last once-daily application of either vehicle (◆), 4 mg (●), 1 mg (■), or 0.25 mg (▲) of arachidonic acid per ear for 1, 2, 3, 5, or 8 d. Means ± S.E.M. are shown.

Marked erythema was noted with all doses of arachidonic acid throughout the 8 d of treatment. The erythema developed within 15 min of the first application and persisted during the 24-h period between applications. The intensity of the erythema response following the second application was not diminished compared to that following the first application, even though the weight increase was greatly reduced following the second application (Fig 1). After the fifth application of 4 mg, the ears exhibited a visible increase in thickness. Crusting of the dorsal surface was also seen by Day 5. These changes were not seen with the lower doses. The edges of the ears treated with 4 mg of arachidonic acid acquired a ragged appearance by this time. The mice were obviously distressed by the application of the highest dose because they groomed the treated ear vigorously. This vigorous grooming may have been responsible for some of the small areas of hemorrhage (scratches?) observed after five or more applications.

Examination of the hematoxylin and eosin stained sections revealed extensive edema in the dermis following the first dose of arachidonic acid (Fig 2*B*). This phenomenon was less marked on subsequent days. From Day 5 onwards, however, the dermis was increased in thickness due to the presence of increased amounts of connective tissue (Fig 2*C*). A marked increase in the thickness of the epidermis was noted after three applications of 4-mg arachidonic acid per ear (Fig 3), and this response reached a plateau after five applications (Figs 2*C* and 3). After five or more applications of the high dose of arachidonic acid, the basal layer of the epidermis had developed marked undulations and resembled human rete ridges (Fig 2*C*).

Small numbers of perivascular neutrophils were noted after the first dose of arachidonic acid, although this is not clearly illustrated in Fig 2*B*. Following the second administration, a few neutrophils could be seen throughout the dermis. From Day 5 onward, neutrophils were also seen in large accumulations between layers of keratinocytes and in the dermis (Fig 2*C*). The MPO content of the ear discs, representing the total number of neutrophils, confirmed the histologic evaluation (Fig 4). Untreated ears had very low levels of MPO, but 1 h after the first application of 4 mg/ear arachidonic acid, a fourfold increase was observed. The second application of 4 mg/ear produced a further slight increase, whereas five doses resulted in a 27-fold increase compared to untreated ears. Applications of acetone did not induce histologically detectable neutrophil infiltration (not shown) and neither one (Fig 4) nor five applications (not shown) increased MPO content.

Toluidine blue stained sections showed marked mast-cell disruption in arachidonic acid treated ears. This disruption appeared to be dose-related and was present throughout the eight days studied. However, it proved impossible to establish an objective scoring system to enable mast-cell disruption to be quantified histologically.

Evans blue accumulation in the ear, an indication of increased capillary permeability and extravasation of plasma into tissues, was very marked after a single dose of arachidonic acid (Fig 5), but was much reduced after the second application (data not shown), as reported by others [5]. After eight daily applications of 4 mg arachidonic acid, plasma extravasation was still reduced compared to the first dose, despite the greater increase in ear weight (Fig 5). These data confirm the histologic impression that the increase in ear weight following the first application of arachidonic acid is mainly due to edema, while the weight increase observed following multiple doses of arachidonic acid cannot be entirely attributed to plasma extravasation. Proliferation of dermis and epidermis presumably contribute significantly to the weight increase at this time. The weight increase observed 24 h after the last of four applications of 4 mg arachidonic acid per ear was similar in magnitude to that ob-

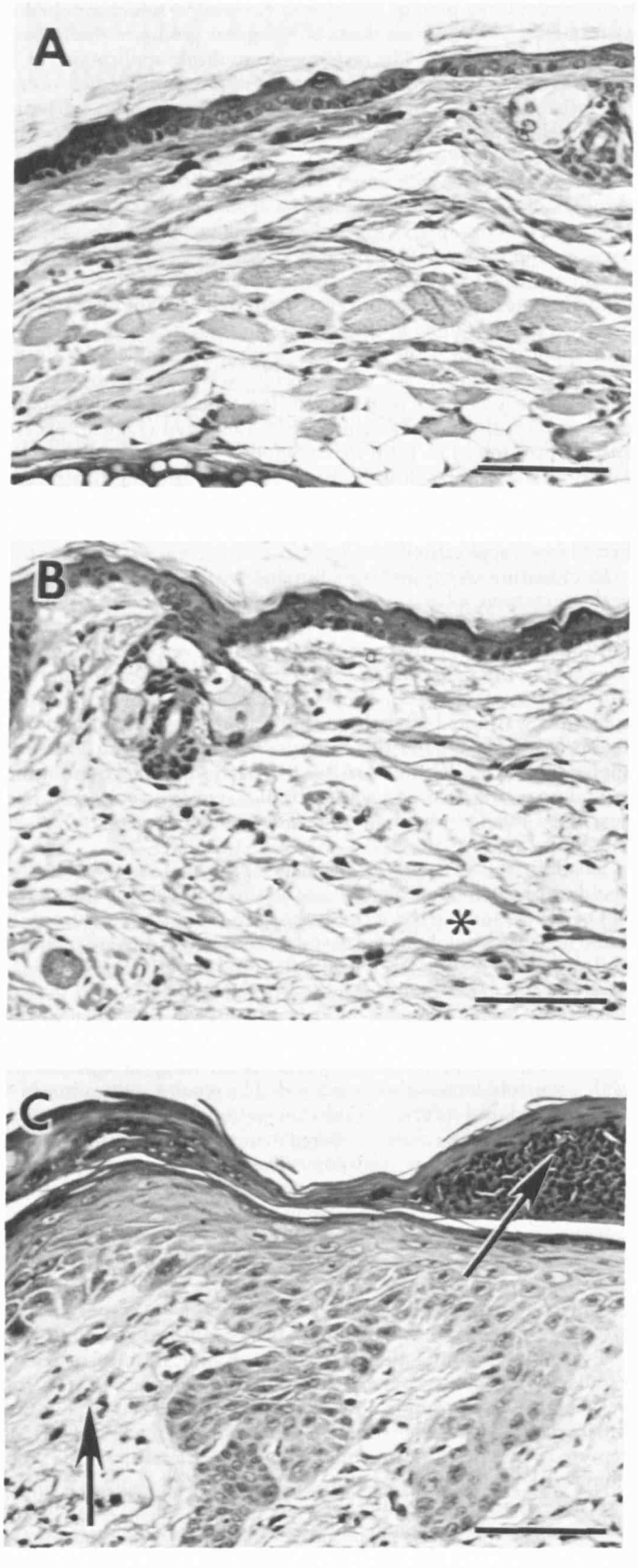

Figure 2. Histologic changes in arachidonic acid treated ears. H & E stained sections of ears treated with *A*: one dose of vehicle; *B*: 1 dose of 4 mg arachidonic acid; *C*: 8 once-daily doses of 4 mg arachidonic acid. Note the dermal edema in section B (*), marked epidermal hyperplasia, and a crust in section *C*. *Arrows* indicate neutrophils. *Bars* = 100 μ.

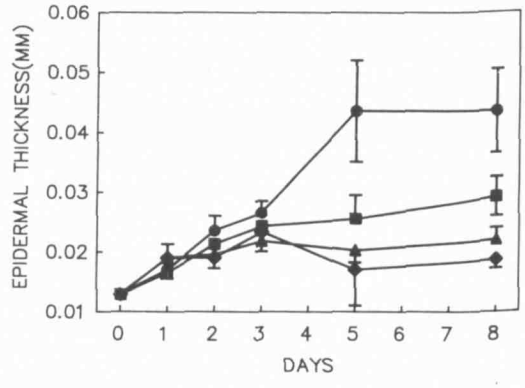

Figure 3. Topical application of arachidonic acid induces increased epidermal thickness. Groups of five mice were killed 1 h after the last once-daily application of either vehicle (◆), 4 mg (●), 1 mg (■), or 0.25 mg (▲) of arachidonic acid per ear for 1, 2, 3, 5, or 8 d. Means ± S.E.M. are shown.

served 1 h after the fifth daily dose (data not shown), indicating that the response to multiple applications persists for at least 24 h, in contrast to the disappearance of the edematous response to the first application [4,5] (Fig 1).

In mast-cell deficient W/W^v mice [17], the ear weight increase in response to the first dose of arachidonic acid was much reduced compared to the wild-type controls (W/+) and CD-1 strain mice (Fig 6). In addition, eight daily doses induced a smaller weight increase and neutrophil accumulation (measured as MPO) compared to the mast-cell sufficient strains (Fig 6); however, no significant differences in epidermal thickness were observed (Fig 6).

Several other fatty acids were compared with arachidonic acid following single or multiple applications of 4 mg/ear to CD-1 strain mice (Fig 7). Arachidonic acid produced the largest weight increase following one or five applications. The magnitude of the weight increase induced by the other fatty acids decreased approximately as the number of double bonds decreased. Arachidonic acid produced the greatest erythema throughout the study, while dihomogamma linoleic acid produced a lesser degree. The other fatty acids produced only slight and intermittent erythema. Only arachidonic acid induced scaling, thickening, and yellow discoloration.

DISCUSSION

The present data, when considered with data from other studies [4,5,7], indicate that the response to daily topical applications of arachidonic acid to the ears of normal mice can be divided into three phases: 1) the first application induces a rapid, but transient, edema accompanied by erythema and accumulation of neutrophils; 2) the second application produces a smaller edema response, although

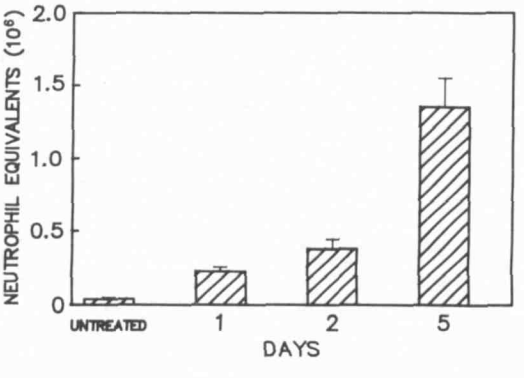

Figure 4. Topical application of arachidonic acid induces neutrophil accumulation. The neutrophil content of ears was measured, as myeloperoxidase accumulation, in groups of at least five mice following 0, 1, 2, and 5 once-daily topical applications of 4 mg arachidonic acid. Means ± S.E.M. are shown.

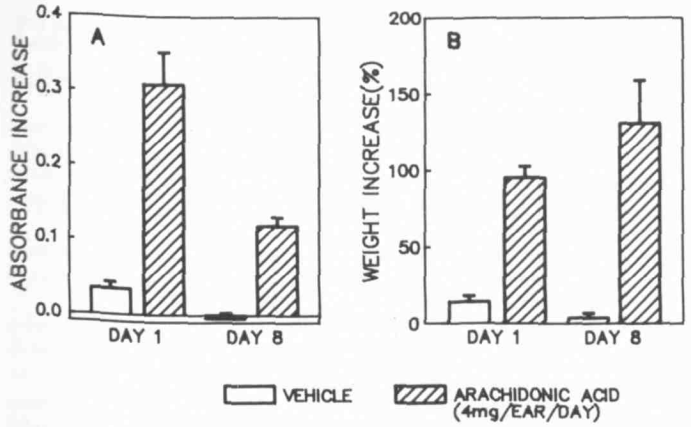

Figure 5. Comparison of arachidonic acid induced edema (Evans Blue accumulation) and ear weight increase. Groups of at least five mice were injected i.v. with Evans Blue immediately prior to the last of either one or eight applications of either vehicle (acetone) or 4 mg arachidonic acid. One hour later the mice were killed and Evans Blue accumulation (*A*) and ear weight increase (*B*) were measured. Means ± S.E.M. are shown.

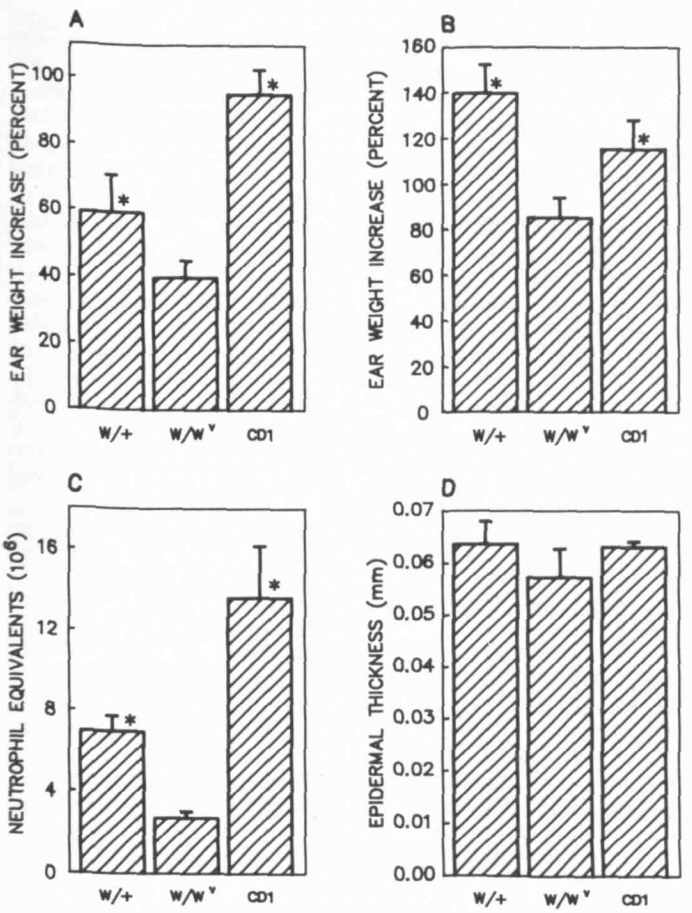

Figure 6. The response to topical application of arachidonic acid in normal (CD1, W/+) and mast cell deficient (W/W^v) mice. Groups of at least five mice were treated with 4 mg arachidonic acid/ear for 1 (*A*) or 8 (*B,C,D*) daily doses. One hour after the last dose ear weight increase (*A,B*), neutrophil accumulation (*C*) and epidermal thickness (*D*) were measured. Means ± S.E.M. are shown. *asterisk:* $p < .05$ compared with W/W^v by analysis of variance and Dunnets multiple comparison test.

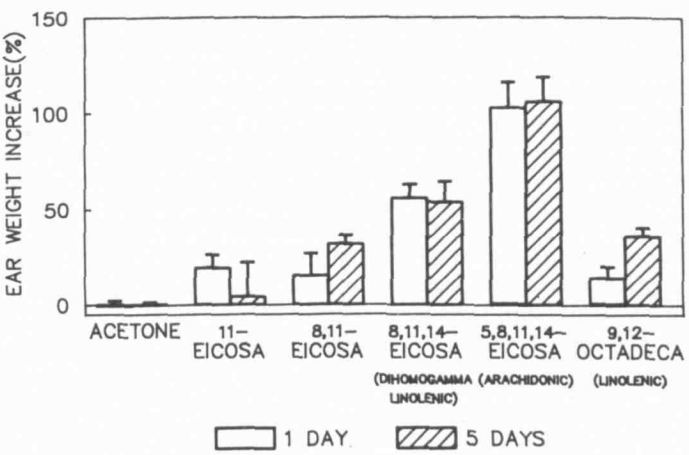

Figure 7. Effects of once-daily applications of different fatty acids on ear weights. Groups of at least five mice were treated with vehicle or one of the fatty acids (4 mg/ear/day) for one or eight once-daily doses. Means ± S.E.M. are shown.

erythema persists and neutrophil accumulation increases; 3) further daily applications maintain the erythema and reduced edema but also induce extensive neutrophil infiltration of both epidermis and dermis, accompanied by proliferation of keratinocytes and dermal connective tissue. Neutrophil accumulation and proliferative changes begin with the first application [5,6], but only after 3 or 4 applications do they dominate the response [14].

The relative specificity among fatty acids for inducing this reaction does suggest that the response is not due to non-specific irritation or a membrane effect induced by common physicochemical properties of fatty acids, although the response is similar to that elicited by some irritants [18]. It is possible that the correlation with the degree of unsaturation is due to the propensity of polyunsaturated fatty acids to form pro-inflammatory products via non-enzymatic air oxidation [10,19].

Nguyen et al [20] have reported results apparently at variance with those described here. When palmitic, linoleic, linolenic, arachidonic, or 5,8,11-eicosatrienoic (ETA) acids were applied to the dorsal skin of hairless mice (approximately 3 mg/day for 4 d), only ETA produced keratinocyte proliferation and neutrophil infiltration. The absence of a response to arachidonic acid could be explained by the numerous methodological differences between the studies; however, it does suggest that ETA, which can be metabolized to leukotrienes [21], although it inhibits prostaglandin synthesis [22], is more potent than arachidonic acid in inducing neutrophil infiltration and keratinocyte proliferation.

There is a wealth of pharmacologic and biochemical data implicating specific metabolities of arachidonic acid in this response. Prostaglandin E_2 (PGE_2) may be the major mediator of erythema [4–6,23] and leukotrienes may be involved in the edema response [4–6,8,23,24]. Of the other fatty acids examined, only dihomogamma linolenic acid is the precursor of a prostaglandin (PGE_1) [25,26] and leukotriene-like compounds [27], perhaps explaining its relative efficacy in inducing erythema and edema. Leukotriene B_4 has been found in arachidonic acid treated ears [6,9,23,24] and has both chemotactic activity for neutrophils [28] and mitogenic activity for keratinocytes [29], thus qualifying it as a potential mediator of these components of the response.

Despite the evidence, summarized above, implicating cyclooxygenase and 5-lipoxygenase products of arachidonic acid, there is a large quantity of pharmacologic data that do not fit easily into this hypothesis. An enormous range of diverse pharmacologic agents, when applied topically, will inhibit the edema response to the first application of arachidonic acid [4–6,8–12]. These data are hard to interpret because topical application produces extremely high local concentrations of the applied material. Therefore, these pharmacologic agents may exert effects at high concentrations that are not

seen at their usual systemic therapeutic doses. Nonetheless, the efficacy of histamine and serotonin antagonists does implicate the release of mast cell amines, consistent with the reduced edema response seen here in mast-cell deficient mice. There is evidence that hydroperoxides or endoperoxides produced during the metabolism of arachidonic acid can induce the release of histamine from mast cells [30]. Therefore, it seems likely that the complex response to topical arachidonic acid is due to interaction of multiple mediators derived either directly from the applied arachidonic acid or from other cells stimulated by arachidonic acid metabolites. A recent report suggests that interleukin 1 may also play a role in these phenomena [31].

The data presented here provide greater understanding of the previously reported responses to topical application of arachidonic acid. Although the proliferative parameters described suggest additional roles for arachidonic metabolites in the pathology of skin [1,2], because mast cells are shown here to mediate a component of the response, care must be taken in attributing responses to the direct effect of arachidonic acid metabolites.

REFERENCES

1. Greaves MW: Leukotrienes in psoriasis and other dermatoses. In: Piper PJ (ed.). The Leukotrienes, Their Biological Significance. Raven Press, New York, 1986, pp. 175–184
2. Kragballe K, Voorhees JJ: Arachidonic acid and leukotrienes in clinical dermatology. Curr Probl Derm 13:1–10, 1985
3. Ruzicka T, Simmet T, Peskar BA, Ring J: Skin levels of archidonic acid derived mediators and histamine in atopic dermatitis and psoriasis. J Invest Dermatol 86:105–108, 1986
4. Young JM, Wagner BM, Spires DA: Tachyphylaxis in 12-0-tetradecanoylphorbol acetate and arachidonic acid-induced ear edema. J Invest Dermatol 80:48–52, 1983
5. Young JM, Spires DA, Redord CJ, Wagner B, Ballaron BS, DeYoung LM: The mouse ear inflammatory response to topical arachidonic acid. J Invest Dermatol 82:367–371, 1984
6. Opas EE, Bonney RJ, Humes JL: Prostaglandin and leukotriene synthesis in mouse ears inflammed by arachidonic acid. J Invest Dermatol 84:253–256, 1985
7. Kuehl FA, Humes JL, Ham EA, Egan EW, Dougherty HW: Inflammation: The role of peroxidase-derived products. In: Samuelsson B, Ramwell PW and Paoletti R (eds.). Advances in Prostaglandin and Thromboxane Research, Vol. 6. New York, Raven Press, 1980, pp. 77–86
8. Carlson RP, O'Neill-Davis L, Lamp B, Chang J, Lewis AJ: Modulation of mouse ear edema by cyclo-oxygenase and lipoxygenase inhibitors and other pharmacologic agents. Agents and Actions 17:197–294, 1985
9. Arner EC, Rule BL, Rudnick MS, Galbraith W: Arachidonic acid-induced ear edema: Correlation of swelling with production of lipoxygenase (LO) products (abstr). Agents and Actions 16:600–601, 1985
10. Chang, J, Doherty NS: Models of inflammatory disease and eicosanoid activities. In: Lewis A, Ackerman N, Otterness I (eds.). Advances in Inflammation Research, Vol 12. New York, Raven Press, 1988, pp 313–316
11. Pignat W, Kienzle R, Bottcher I: How specific is the arachidonic acid-induced mouse ear edema for lipoxygenase (LO) and cyclo-oxygenase (CO)-inhibitors? Agents and Actions 19:368–370, 1986
12. Crummey A, Harper GP, Boyle EA, Mangan FR: Inhibition of arachidonic acid-induced ear edema as a model for assessing topical anti-inflammatory compounds. Agents and Actions 20:69–76, 1987
13. Nashed W, Rovee DT, Gander RJ: Topical acylaminophenols. U.S. Patent 4,329,366. 1982
14. Nordlund J: Chemical agents which mimic the effects of ultraviolet irradiation on the epidermis: A possible role for oxidation of arachidonic acid in expression of surface markers on epidermal cells. In: Parrish J (ed.). The Effects of Radiation of the Immune System. Johnson & Johnson, 1984, pp. 161–179
15. Lundberg C, Arfors K-E: Polymorphonuclear leukocyte accumulation in inflammatory dermal sites as measured by ^{51}Cr-labelled cells and myeloperoxidase. Inflammation 7:247–255, 1983
16. Bradley PP, Priebat DA, Christensen RD, Rothstein G: Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. J Invest Dermatol 78:206–209, 1982
17. Ponvert C, Galoppin L, Scheinmann P, Canu P, Burtin C: Tissue histamine levels in male and female mast cell deficient mice (W/W^v) and their littermates ($W^v/+$, $W/+$ and $+/+$). Agents and Actions 17:1–4, 1985
18. Patrick E, Burkhalter A, Maibach H: Recent investigations of mechanisms of chemically induced skin irritation in laboratory mice. J Invest Dermatol 88 (suppl):24–31, 1987
19. Cunningham FM, Woollard PM, Camp RDR: Proinflammatory properties of unsaturated fatty acids and their monohydroxy metabolites. Prostaglandins 30:497–509, 1985
20. Nguyen TT, Ziboh VA, Uematsu S, McCullough JL, Weinstein G: New model of a scaling dermatosis: Induction of hyperproliferation in hairless mice with eicosa-5,8,11-trienoic acid. J Invest Dermatol 76:384–387, 1981
21. Orning L, Bernstrom K, Hammarstrom S: Formation of leukotrienes E_3, E_4 and E_5 in rat basophilic cells. Eur J Biochem 120:41–45, 1981
22. Ziboh VA, Vanderhoek JY, Lands EM: Inhibition of sheep vesicular gland oxygenase by unsaturated fatty acids from skin of essential fatty acid deficient rats. Prostaglandins 5:233–240, 1974
23. Blackham A, Norris AA, Wood BE: Role of neutrophils and arachidonic acid metabolites in experimental models of ear inflammation in mice. Br J Pharmacol 86:786P, 1985
24. Harris RR, Mackin WM, Ackerman NR: Cellular and biochemical characterization of the inflammatory effects of topically applied arachidonic acid (AA) to murine skin. Clin Res 34:754A, 1986
25. Williams TJ: Vascular responses and their suppression. In: Bonta IL, Bray MA, Parnham MJ (eds.) Handbook of Inflammation, Vol. 5. Elsevier, Amsterdam, 1985, pp. 49–60
26. Bergstrom S, Danielsson H, Klenberg D, Samuelsson B: The enzymatic conversion of essential fatty acids into prostaglandins. J Biol Chem 239:PC4006–PC4008, 1964
27. Jubiz W, Nolan G: Leukotrienes produced by incubation of dihomo-γ-linolenic acid with human polymorphonuclear leukocytes. Biochem Biophys Res Commun 114:855–862, 1983
28. Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith MJH: Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature 286:264–265, 1980
29. Kragballe K, Desjarlais L, Voorhees JJ: Leukotriene B_4, C_4, and D_4 stimulate DNA synthesis in cultured human keratinocyte cultures. Br J Dermatol 113:43–52, 1985
30. Masini E, Giannella E, Bani-Sacchi T, Fantozzi R, Palmerani B, Mannaioni PF: Histamine release from serosal mast cells by intermediate products of arachidonic acid metabolism. Agents and Actions 20:202–205, 1987
31. Ristow H-J: A major factor contributing to epidermal proliferation in inflammatory skin diseases appears to be interleukin 1 or a related protein. Proc Natl Acad Sci USA 84:1940–1944, 1987