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The Permeability Barrier in Essential Fatty Acid Deficiency: Evidence for a Direct Role for Linoleic Acid in Barrier Function

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Essential fatty acid (EFA) deficient rodents demonstrate abnormal epidermal permeability barrier function and differentiation, defects which can be corrected by either topical or systemic administration of linoleic acid. Since linoleic acid is a precursor of prostaglandins, correction of the defect in barrier function may either reflect a prostaglandin-mediated return toward normal epidermal differentiation, or, instead, a direct effect of linoleic acid. To test these possibilities severely EFA-deficient mice were pretreated daily with indomethacin and/or 5,8,11,14-eicosatetrayeonic acid, and then placed on normal (linoleic acid-supplemented) diets. Endogenous formation of prostaglandin E_2 was determined by thin-layer chromatography after transformation into prostaglandin B_2 with ethanolic-hydrochloric acid. Animals treated with both indomethacin and TYA demonstrated substantial reductions in prostaglandin E_2 levels in liver and skin. Animals replenished with linoleic acid invariably demonstrated a rapid return of barrier function toward normal whether or not they were blockaded, while non-replenished animals, with or without inhibition of prostaglandin biosynthesis, demonstrated continued deterioration in barrier function. In other experiments, topically applied linoleic acid rapidly reversed the defect in barrier function at the sites of application prior to systemic correction of the EFA deficient state. These results suggest that: (1) defective cutaneous barrier function in EFA deficiency can be corrected locally without prior systemic reversal of the deficiency state; and (2) that linoleic acid may play a direct role in the epidermal permeability barrier independent of its role in prostaglandin metabolism.

Exclusion of linoleic acid from the diet of weanling rodents results in a characteristic, multisystem deficiency state—termed essential fatty acid deficiency (EFAD) (reviewed in references 1 and 2). In EFAD the skin exhibits characteristic abnormalities of keratinization and permeability barrier function [2–7]. Both the skin and multisystem disease in EFAD can be corrected by topical applications of linoleic acid [8–10]. Linoleic acid could repair the skin signs of EFAD by increasing endogenous generation of arachidonic acid and its prostaglandin metabolities, which in turn, would normalize both the keratinization process and barrier function [5,11]. Or, as an increasing body of evidence now asserts, linoleic acid may directly affect barrier function, without the necessity of its further metabolism to arachidonic acid and its metabolites [7].

In this study, we have shown: (1) that both free and esterified linoleic acid are capable of improving barrier function locally, without prior correction of the systemic deficiency state, and (2) that barrier function can be corrected over pharmacologic blockade of both the lipoxygenase and cyclo-oxgenase pathways of arachidonic acid metabolism.

METHODS

Chemicals and Solvents

Chemicals: The following chemicals, from Sigma Chem. Co., St. Louis, Mo., were utilized: indomethacin (1-{p-chlorobenzoyl}-5-methoxy-2-methyl-indole-3-acetic acid), crystalline; cis-9-cis-12-octadecadienoic acid (linoleic acid), grade III, approx. 99% pure; and cis-9-octadecanoic acid (oleic acid), approx. 99% pure.

Tetrayenoic acid (TYA) was kindly donated by Hoffman-LaRoche Inc., Nutley, N.J.

Solvents: Chloroform and methyl alcohol, both analytical grades, were purchased from Mallinckrodt, Inc., Paris, Ky. Benzene, instra-analyzed, was obtained from J. T. Baker Chem. Co., Phillipsburg, N.J.

Animals and Diets

Normal weanling (3-4 weeks old) ICR and hairless (strain HRS/J from Jackson Labs., Bar Harbor, Maine) mice were put on either linoleic acid-free or control diets, composed and prepared as previously described [12]. Animals were maintained at 27–29°C and given water *ad libitum*. After 8 to 12 weeks all animals on deficient diets exhibited cutaneous signs of severe EFAD.

Measurement of Transepidermal Water Loss

Water loss was measured by passing a stream of dry nitrogen through a flow cell fitted with a cup (0.21 cm^2 orifice) held against the ventral surface of the ear [13]. The moisture content in the effluent was then detected by means of an electrolytic water analyzer (Manufacturers Engineering and Equipment Corp., Warrington, PA.). All measurements of transepidermal water loss (TEWL) were made bilaterally at the same time each day, and under identical conditions of environmental temperature and humidity. Although water loss varied from animal to animal, for a given animal the water loss on contralateral ears correlated closely.

The results of the transepidermal water loss measurements in the various groups were compared statistically by Student's *t*-test.

Lipid Extraction and Analysis

At the termination of experiments, full-thickness skins and livers of both control and experimental animals were removed under ether anesthesia. Tissues were minced into 1-to-2 mm^3 pieces in iced phosphate-buffered saline and then transferred to a ground glass homogenizer and extracted overnight with chloroform: methanol:water (2:4:1.6, v/v/v) using the procedure of Bligh and Dyer [14] at a ratio of approximately 1 ml of solvent to 10 to 15 mg of tissue [15]. After thorough homogenization, the suspension was re-extracted with fresh solvent and agitated in a modified Burrel wrist-action shaker for an additional 30 min at room temperature. The extraction media were converted to a 2-phase system with equal volumes of chloroform and water, again according to Bligh and Dyer, and the phases were separated by low-speed centrifugation. Subsequently, the upper aqueous phases were discarded and the combined lower phases washed with clean upper phase solvent from the 2-phase system (chloroform:methanol:water, 4:4:3.6, v/v/v). The combined lower phases were then

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Abbreviations:
EFAO: essential fatty acid deficiency
TEWL: transepidermal water loss
TYA: tetrayenoic acid

evaporated to dryness in a nitrogen atmosphere, weighed, re-suspended in absolute benzene, and stored at −20°C.

We obtained fatty acid methyl esters of total hepatic lipids as previously described [4]. The quantities of fatty acid methyl esters in control and in essential fatty acid-deficient animals were expressed as molar ratios of various fatty acid methyl esters to levels of palmitic acid, which varied only slightly in control *versus* deficient animals (reference 4, 5.55 *versus* 5.06). A ratio of eicosatrienoic to arachidonic acid of greater than 0.4 is considered diagnostic of essential fatty acid deficiency [16]. After 8 weeks on the deficient diet, hepatic fatty acids exhibited distinctive changes in fatty acid composition [4].

Thin-Layer Chromatography of PGE_2 in Skin and Liver Specimens

Since PGE_2 is a major prostaglandin formed from arachidonic acid in skin, we decided to assay for its level as a measure of arachidonic acid transformation into PGs. Similar assays were carried out with liver specimens. To attain this objective specimens of skin and liver were homogenized in a mixture of chloroform:methanol (2:1, v:v). After an aliquot was first taken for protein determination, the remaining homogenate was filtered through glass wool to remove debris. The total lipid extract, with a known amount of ^{14}C-PGE_2 added as a standard, was then evaporated to dryness in a rotary evaporator. The residue was dissolved in a small volume of chloroform:methanol (1:1, v/v) and run on activated silica gel G coated thin-layer chromatography plates in chloroform:methanol:acetic acid:water (90:8:1:0.8, v/v/v/v). The developed plates were then placed in a scanner, and the silica gel portions corresponding with ^{14}C-PGE_2 were scraped into sintered funnels and the prostaglandins eluted with chloroform:methanol (1:1, v/v).

For further identification, the PGE_2 eluted from the various samples and the ^{14}C-PGE_2 was transformed into PGB_2 by treatment of each sample with 3 ml of 0.5 N NaOH in 50% aqueous ethanol at room temperature for 30 min as reported previously [17]. Known amounts of nonradioactive PGE_2 were treated similarly. After 30 min, the samples were diluted with water and then acidified with 6 N HCl to pH 2-3. The samples were next extracted three times with 10 ml of absolute dichloromethane and the combined extracts were evaporated to dryness under nitrogen. Under these conditions, the E prostaglandins form products containing the $\Delta^{8(12),13}$−9 keto chromophore by elimination of the 11-hydroxy group and isomerization of the resulting double bond [18,19]. The dried samples were dissolved in minimal amounts of chloroform:methanol (1:1, v/v), applied to activated silica gel G plates, and developed in ether:acetic acid (100:2, v/v). The silica gel portion corresponding with ^{14}C-PGB_2 was eluted as above and dried under nitrogen.

The PGB_2 residues were dissolved in ethanol and the absorbance read at 278 nm in a Gilford, Model 240 spectrophometer. Estimations of PGB_2 in samples were determined after comparison with a standard curve of known amounts of PGB_2. Corrections for loss were made with knowledge of the ^{14}C-PGE_2 remaining after the entire procedure. Results are expressed as nanograms of PGB_2 per mg of tissue protein.

Topical Application of Lipids

Three separate experiments were performed in which deficient and control (corn oil-supplemented) ICR animals, maintained on EFAD diets for 8-12 weeks, were divided into 2 groups of 4 to 12 animals each. All animals were maintained at 27 to 29°C in a humidified environment during these experiments.

In the first set of experiments, half of the control and half of the deficient animals received twice-daily applications of corn oil (20 μL) or linoleic acid (6 mg) to the ventral surface of the right ear, while the contralateral ear was treated similarly with twice-daily applications of either olive oil (20 μL) or oleic acid (6 mg). The purity of each fatty acid was checked by thin-layer chromatography against known standards, and each was dissolved in propylene glycol:ethanol prior to application (3:7, v/v) as previously described [20]. Care was taken to insure that the solutions were spread evenly and did not run. Transepidermal water loss determinations were made at least 6 hr after the last prior application of oil, and immediately prior to the next topical treatment. Measurements were taken from the untreated dorsal surface of the right ear and on corresponding surfaces of the left ear. At the end of each 6 day experiment, the livers of animals in each of the experimental groups were excised, pooled, extracted with Bligh-Dyer solvents, and analyzed for extent of essential fatty acid deficiency by gas liquid chromatography, as described above.

Replenishment/Blockade Experiments

Two separate experiments were performed in which ICR mice, exhibiting signs of severe EFAD, were randomly divided into groups which received indomethacin, tetrayenoic acid (TYA), or both (see below). In one experiment half the animals in each group were refed with control diets (linoleic acid-supplemented), while the other half remained on deficient diets. In the second experiment, replenishment was accomplished both by resumption of the control diets and by topical applications of 12 mg of linoleic acid. A control group fed and topically treated only with oleic acid served as the nonreplenished control group.

Mice were pretreated for 3 days, and then daily throughout the experiment, with intraperitoneal injections of indomethacin (15 mg/kg body wt.) and/or tetrayenoic acid (30 mg/kg body wt). This dose of indomethacin is 30 times higher than that recently demonstrated to block prostaglandin synthesis in EFAD rats and about half the dose shown to decrease prostaglandin synthesis by 98% in guinea pigs (references cited in 21). The dose of TYA is comparable to that recently shown to inhibit arachidonic acid to PG conversion in mouse skin *in vitro* [22,23]. Injections contained final concentrations of 0.06% TYA and/or 0.03% indomethacin in 24% v/v dimethylsulfoxide (DMSO) and 24% serum in normal saline. The serum for these experiments had been previously obtained by exsanguinating and pooling the body of severely EFAD mice. An additional, control group of EFAD mice received daily injections with the control solution containing equivalent amounts of DMSO, serum, and saline.

Transepidermal water loss was measured daily for 4 days over the ventral surface of the ears of animals in all groups. At the termination of the experiments the livers of animals from each group were pooled, extracted, and analyzed by gas liquid chromatography for the extent of essential fatty acid deficiency [4], and by thin-layer chromatography to determine the degree of inhibition of the cyclo-oxygenase pathway.

RESULTS

Topical Replenishment Experiments—Linoleic vs. Oleic Acid

Gross changes were apparent in the appearance of linoleic acid vs. oleic acid-treated ears during the topical replenishment experiments. On days 1, 2, and 3 ears treated with linoleic acid appeared more normal than did the contralateral ears treated with oleic acid, but by day 4 these differences were imperceptible.

These gross changes were paralleled by changes in barrier function, again differing depending on the lipid treatment. As seen in Fig 1 and 2 topical applications of olive oil or oleic acid produced actual deterioration in barrier function. In contrast, simultaneous application of corn oil or linoleic acid to the contralateral ear of the same animal produced a partial correction in barrier function in that ear within 2 to 4 days. After multiple applications, however, the differences in water loss between the 2 groups began to converge, presumably because sufficient linoleic acid was absorbed eventually to systemically correct EFAD (Fig 2).

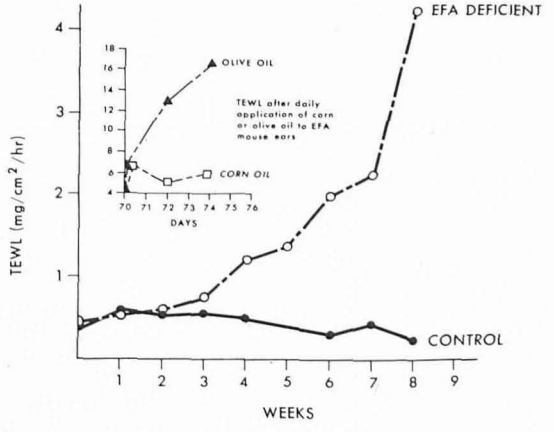

FIG 1. Transepidermal water loss deteriorates as linoleic acid is withheld from diet. However, when esterified linoleic acid in the form of corn oil is applied to the right ear, and esterified oleic acid in the form of olive oil to the left ear, a rapid divergence in water loss measurements is obvious.

Replenishment Over Arachidonic Acid Blockade

In order to assess whether linoleic acid plays a direct role in barrier function or a role secondary to correction of aberrant prostaglandin synthesis, severely EFAD animals were replenished while under treatment with indomethacin and/or TYA. As seen in Fig 3, whether or not animals were treated with these pharmacologic agents, either systemically or topically administered linoleic acid, corrected the defect in TEWL. Thin-layer chromatography of hepatic and skin lipid extracts confirmed that both TYA and indomethacin produced a profound reduction in prostaglandin biosynthesis (Table).

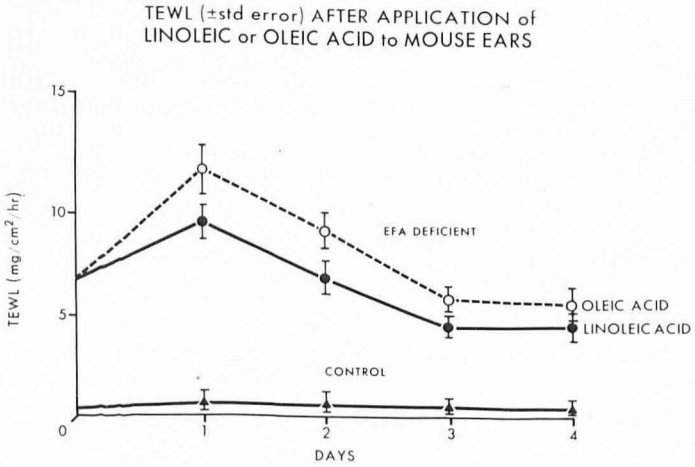

FIG 2. Transepidermal water loss measurements in severely EFAD animals after applications of pure linoleic acid and oleic acid to contralateral ears of same animal. Note that initially both acids transiently perturb barrier function, but linoleic acid does so to a lesser extent, and begins to correct barrier function by 2 days. The differences between the 2 ears are not statistically significant by 4 days, indicating possible systemic correction by this time. Control ears are not effected by topical applications of either acid. In this experiment, units of transepidermal water loss were recorded as raw data (units).

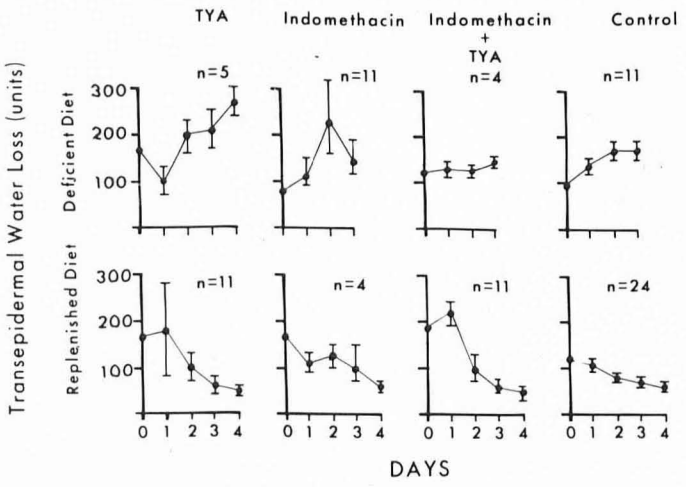

FIG 3. Correction of barrier function in EFAD mice by dietary replenishment over arachidonic acid blockade (see text). Note that in upper row (non-replenished controls), barrier function of animals in all groups stayed the same or deteriorated substantially. In contrast, graphs in the lower row illustrate that barrier function is corrected rapidly by dietary replenishment in all groups, regardless of the type of blockade of arachidonic acid metabolism (see text).

Effect of pharmacologic blockade of arachidonic acid metabolism on skin and hepatic prostaglandin B_2 levels

Tissue	Treatment	Diet	PGB_2 (ng/mg protein)	(% change)[a]
Skin	Vehicle only	Deficient	4.4	(–)
		Replenished	270.0	(0%)
	TYA alone	Deficient	4.0	(–)
		Replenished	46.0	(–82.9%)
	Indo alone	Deficient	Not done[b]	(–)
		Replenished	17.1	(–93.6%)
	Indo + TYA	Deficient	3.75	(–)
		Replenished	46.8	(–82.6%)
Liver	Vehicle only	Deficient	8.3	(–)
		Replenished	583.3	(0%)
	TYA alone	Deficient	3.5	(–)
		Replenished	33.0	(–94.3%)
	Indo alone	Deficient	Not done[b]	(–)
		Replenished	35.4	(–93.8%)
	Indo + TYA	Deficient	4.82	(–)
		Replenished	30.2	(–94.8%)

[a] Expressed as percent reduction of PGB_2 in each group in comparison to vehicle-treated, replenished group.

[b] There were no surviving mice in this group.

DISCUSSION

Linoleic Acid in the Epidermal Permeability Barrier

Linoleic acid deprivation leads to a characteristic deficiency syndrome (EFAD), characterized in rodents by abnormal keratinization and enhanced transepidermal water loss [3,6,7]. EFA epidermis is thickened and exhibits increased DNA synthesis [5,6]. It has been suggested that the increased porosity of EFAD epidermis is due to aberrant keratinization, which is, in turn, leads secondarily to release from prostaglandin inhibition of cell division. But abundant evidence is now available which indicates that the abnormal keratinization and defective barrier function of EFAD epidermis are divergent phenomena. Recent work has demonstrated defective deposition of intercellular, neutral lipids in EFAD mouse stratum corneum and stratum granulosum [4]. That the important lipid for barrier function is linoleic acid itself, and not prostaglandins, is supported by several observations: Although topically applied prostaglandins reduce the scaling of EFAD epidermis [11], they are less effective than linoleic acid in reducing defective barrier function [24]. Topical linoleic acid, but not oleic acid, also repairs abnormal barrier function in detergent-treated skin [25]. Moreover, topical linoleic acid corrects EFAD barrier function when the biosynthesis of prostaglandins is inhibited [26], and decreases EFAD hyperplasia without return of prostaglandin levels to normal [20]. Finally, linoleic acid-containing lipids have been found to both impede water loss across experimental burn wounds and as a monomolecular film *in vitro* [27].

The studies reported here lend further support to this emerging hypothesis. First, topically applied linoleic acid rapidly corrected the defect in barrier function in EFAD mice, while oleic acid caused actual deterioration in the same animal. Although the more gradual correction of barrier function on the oleic acid-treated side presumably reflects sufficient systemic absorption of linoleic acid to initiate systemic reversal of the deficiency state, direct evidence of this was not obtained in these experiments. The rapidity (1 day) with which linoleate corrected the barrier abnormality suggests a direct local effect, but it is theoretically possible, though unlikely, that sufficient prostaglandins are generated locally [23,28,29] to normalize differentiation and barrier function. In contrast, oleic acid caused deterioration in barrier function. Although the cause of this effect remains unknown, it may reflect increased bioavailability of monoenoic acid precursors for conversion to eicosatrienoic acid, which, in turn, might further aggravate barrier function. In 2 recent studies topical linoleate did not effect

arachidonic acid levels after 5 days [30], and even after 14 days prostaglandin levels did not return to normal [20]. Thus, it is likely that the rapid, localized return to normal observed here reflects a specific effect of linoleic acid rather than a secondary response to normalized prostaglandin levels.

Second, topical or systemically administered linoleic acid corrected defective barrier function, even over substantial pharmacologic blockade of prostaglandin biosynthesis. These studies extend earlier observations, where the barrier was corrected by linoleate over indomethacin blockade [26], but in the earlier study much lower (0.5 mg/kg) and more infrequent (every other day) doses of indomethacin were given than in this study. Furthermore, in this study we also attempted to inhibit the lipoxygenase pathway, in addition to the cyclo-oxygenase pathway, by administering tetrayenoic acid, an effective inhibitor of arachidonic acid metabolism [21]. This distinction is important because certain hydroxy fatty acid metabolites of the lipoxygenase pathway of arachidonic acid metabolism may be regulators of epidermal differentiation [31]. Although these experiments lend further support for a direct role of linoleic acid, the possibility still remains that the low levels of prostaglandins that continue to be generated (Table, reference 20) may still be sufficient to normalize epidermal differentiation.

In view of the general importance of lipids for barrier function, and for the seeming special role for linoleic acid, it is curious that there is not an overbundance of linoleate in stratum corneum lipids [15,32,33]. However, linoleic acid is found in disproportionate large amounts in certain mouse, porcine, and human epidermal sphingolipids [32–34], which have been implicated as being of importance for the maintenance of the intercellular lipid bilayers in the stratum corneum [32,33]. Studies are currently underway in this laboratory to determine whether these compounds are altered specifically in EFAD.

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REFERENCES

1. Alfin-Slater RB: Essential fatty acids—Recent developments, Lipid Pharmacology, Vol. II. 1976, p 43–82
2. Holman RT: Essential fatty acids in human nutrition, International Symposium on Function and Biosynthesis of Lipids. Adv Exp Med Biol 83:515–534, 1977
3. Basnajake V, Sinclair HM: The effects of deficiency of essential fatty acids upon the skin, Biochemical Problems of Lipids. Edited by G Popjak, E LeBreton. London, Butterworth, 1956, pp 476–484
4. Elias PM, Brown BE: The mammalian cutaneous permeability barrier: Defective barrier function in essential fatty acid deficiency correlates with abnormal intercellular lipid deposition. Lab Invest 39:574–583, 1978
5. Lowe NJ, Stoughton RB: Essential fatty acid deficient hairless mouse: A model of chronic epidermal hyperproliferation. Br J Dermatol 96:155–162, 1977
6. Menton DN: The effects of essential fatty acid deficiency on the skin of the mouse. Am J Anat 122:337–356, 1968
7. Prottey C: Essential fatty acids and the skin. Br J Dermatol 94: 579–587, 1976
8. Friedman Z, Shochat SJ, Maisels J, Marks KH, Lamberth EL: Correction of essential fatty acid deficiency in newborn infants by cutaneous application of sunflower-seed oil. Pediatrics 58:630–634, 1976
9. Press M, Hartop PJ, Prottey C: Correction of essential fatty acid-deficiency in man by the cutaneous application of sunflower-seed oil. Lancet 1:597–599, 1974
10. Prottey C, Hartop PJ, Press M: Correction of the cutaneous manifestations of essential fatty acid deficiency in man by the application of sunflower-seed oil to the skin. J Invest Dermatol 64: 228–234, 1975
11. Ziboh VA, Hsia SL: Effects of prostaglandin E_2 on rat skin: Inhibition of sterol ester biosynthesis and clearing of scaly lesions in essential fatty acid deficiency. J Lipid Res 13:458–467, 1972
12. Williams MA, Tamai KT, Hincenbergs I, McIntosh DJ: Hydrogenated coconut oil and tissue fatty acids in EFA-depleted and EFA-supplemented rats. J Nutr 102:847–856, 1972
13. Thiels FAJ, Schutter K: Moisture meters for measuring the water balance of the human skin. Proc Toilet Goods Assoc 40:20, 1963
14. Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. Canad J Biochem Physiol 37:911–917, 1959
15. Elias PM, Goerke J, Friend DS: Mammalian epidermal barrier lipids: Composition and influence on structure. J Invest Dermatol 69:535–546, 1977
16. Fulco AJ, Mead JF: Metabolism of essential fatty acids. VII. Origin of 5,8,11 eicosatrienoic acid in the fatty acid deficient rat. J Biol Chem 234:1411–1416, 1959
17. Ziboh VA: Biosynthesis of prostaglandin E_2 in human skin: Subcellular localization and inhibition by unsaturated fatty acids and antiinflammatory drugs. J Lipid Res 14:377–384, 1973
18. Bergstrom S, Dressler F, Krabisch L, Ryhage R, Sjovall J: The isolation and structure of a smooth muscle stimulating factor in normal sheep and pig lungs. Prostaglandin and related factors. 9. Ark Kemi 20:63–66, 1963
19. Bergstrom S, Ryhage R, Samuelsson B, Sjovall S: Prostaglandin and related factors. 15. The structures of prostaglandin E_1, $F_{1\alpha}$ and $F_{1\beta}$. J Biol Chem 238:3555–3546, 1963
20. Lowe NJ, DeQuoy PR: Linoleic acid effects on epidermal DNA synthesis and cutaneous prostaglandin levels in essential fatty acid deficiency. J Invest Dermatol 70:200–203, 1978
21. Flower RJ: Drugs which inhibit prostaglandin biosynthesis. Pharmacol Rev 26:33–67, 1974
22. Hammerstom S, Lindgren JA, Marcelo C, Duell EA, Anderson TF, Voorhees JJ: Arachidonic acid transformations in normal and psoriatic skin. J Invest Dermatol 73:180–193, 1979
23. Sadowski JA: Metabolism and release of arachidonic acid by primary mouse epidermal cell cultures. Fed Proc 38:752a, 1979
24. VanDorp DA: Essential fatty acids and prostaglandins, 24th International Congress of Pure and Applied Chemistry, Vol. 2. London, Butterworth, 1974, p 117
25. Prottey C, Hartop PJ, Black JG, McCormack JI: The repair of impaired epidermal barrier function in rats by the cutaneous application of linoleic acid. Br J Dermatol 94:13–21, 1976
26. Prottey C: Investigaion of functions of essential fatty acids in the skin. Br J Dermatol 97:29–38, 1977
27. Jelenko C, Wheeler ML, Scott TH: Studies in burns. X. Ethyl linoleate: The water-holding lipid of skin. A. The evidence. J Trauma 12:968–977, 1972
28. Jonsson CE, Anggard E: Biosynthesis and metabolism of prostaglandin E_2 in human skin. Scand J Clin Lab Invest 29:289–296, 1972
29. Ziboh VA: Biosynthesis of prostaglandin E_2 in human skin: Subcellular localization and inhibition by unsaturated fatty acids and anti-inflammatory drugs. J Lipid Res 14:377–384, 1973
30. Hartop PJ, Prottey C: Changes in transepidermal water loss and the composition of epidermal lecithin after application of pure fatty acid triglycerides to the skin of essential fatty acid-deficient rats. Br J Dermatol 95:255–264, 1976
31. Hammerstrom S, Hamberg M, Sammuelsson B, Duell EA, Stawiski M, Voorhees JJ: Increased concentrations of nonesterified arachidonic acid, 12, 1-hydroxy-5,8,10,14-eicosatrienoic acid, prostaglandin E_2, and prostaglandin F_{2a} in epidermis of psoriasis. Proc Natl Acad Sci 82:5130–5134, 1975
32. Elias PM, Brown BE, Goerke J, Fritsch P, Gray GM, White RJ: Localization and composition of lipids in neonatal mouse stratum granulosum and stratum corneum. J Invest Dermatol 73:339–348, 1979
33. Gray GM, White RJ: Glycosphingolipids and ceramides in human and pig epidermis. J Invest Dermatol 70:336–341, 1978
34. Gray GM, White RJ, Majer RJ: 1-(3′-0′acyl)-β-glucosyl-N-dihydroxy-pentatriacontadienoylsphingosine, a major component of the glucosylceramides of pig and human epidermis. Biochim Acta 528:127–137, 1978