Effects of prostaglandin E$_2$ on rat skin: inhibition of sterol ester biosynthesis and clearing of scaly lesions in essential fatty acid deficiency

Vincent A. Ziboh and S. L. Hsia
Departments of Dermatology and Biochemistry, University of Miami School of Medicine, Miami, Florida 33136

Abstract  Severe scaly lesions in the skin, especially in the feet and tail, of the rat were induced by feeding a diet deficient in essential fatty acids (EFA). Analysis of the fatty acids in skin lipids of these EFA-deficient rats showed a marked increase of monounsaturated acids (16:1 and 18:1) and eicosatrienoic acid (20:3), with concomitant decreases of dienoic acid (18:2) and tetraenoic acid (20:4). Topical application of prostaglandin E$_2$ (PGE$_2$) to the scaly lesions resulted in clearance of the lesions, but did not significantly alter the composition of fatty acids in the skin. Intraperitoneal injection of PGE$_2$ had no observable effect on the skin lesions. Furthermore, incubation of skin specimens from the EFA-deficient rats with $^{14}$C-labeled glucose showed a 4-5-fold increase of incorporation of glucose carbon into lipid fractions, particularly the sterol esters, and a 3-4-fold increase in pentose cycle activity. Addition of PGE$_2$ to the incubation mixture resulted in approximately 70% inhibition of sterol ester biosynthesis by skin of the EFA-deficient rats. These results suggest that the effects of PGE$_2$ in clearing the scales may be associated with its inhibitory effect on abnormal sterol esterification in the skin of the EFA-deficient rats.

Supplementary key words  safflower oil  coconut oil  polyunsaturated fatty acids  eicosatrienoic acid  neutral lipids  polar lipids  sterol esters  keratinization

The nutritional requirement of mammalian species for certain fatty acids was first recognized in young rats and described by Burr and Burr (1, 2). These investigators demonstrated that rats maintained on a fat-free diet over a long period developed an abnormality characterized by scaliness of the dorsal skin, the feet, and the tail, which later became necrotic. These signs were accompanied by growth retardation, impaired fertility, increased water consumption, and diminished urine production. Burr and Burr proposed that linoleic and possibly linolenic acids were essential fatty acids (EFA). The typical skin lesions therefore serve to reflect a derangement of metabolism associated with EFA deficiency. Apart from the macroscopic symptoms characterized by severe scaling in the skin, very little is known about the biochemical events occurring in this tissue in EFA deficiency.

Although arachidonic acid is not a normal component in vegetable fats, it is considered to be the principal unsaturated fatty acid required by the animal organism (3, 4). This line of thought is strengthened by reports from several investigators (5–8) who have shown that linoleic and γ-linolenic acids undergo transformations in the animal organism to give arachidonic acid. Widmer and Holman (9) reported increased levels of arachidonate in the heart and liver of fat-deficient rats fed diets supplemented with linoleic acid. In control rats that received stearate and olate, no increase in polyunsaturated acids in these organs could be detected. Greenberg et al. (10) reported that methyl arachidonate pos-
sessed a biopotency three to five times that of linoleic acid in correcting EFA deficiency.

Recent studies have demonstrated that some of the EFA can be transformed enzymatically into prostaglandins by homogenates of vesicular glands from sheep (11–13) and adipose tissue of rats (14). A study in our laboratory has also demonstrated that arachidonic acid is transformed into PGE2 by homogenates of rat skin (15). It is therefore interesting to investigate if prostaglandins play any role in the syndrome of EFA deficiency.

This communication describes our observations that PGE2 applied topically can clear the cutaneous lesions induced by feeding the rat a diet deficient in EFA. The facts that arachidonic acid is a precursor for the biosynthesis of PGE2 and that PGE2 exerts a curative effect on skin lesions in EFA deficiency suggest that the biochemical action of PGE2 in the skin may in some way mediate the normal process of keratinization.

In further experiments we found, among other abnormalities, that the skin of the EFA-deficient rats synthesizes greater amounts of lipids from glucose than normal, especially the sterol esters. We also found that PGE2 markedly inhibits the biosynthesis of sterol esters by skin of the EFA-deficient rats. It is tempting to associate the curative effects of PGE2 with its inhibitory effect on the synthesis of sterol esters in the skin. To our knowledge, this is the first observation of a biochemical event relating PGE2 to EFA deficiency.

**MATERIALS AND METHODS**

Constituents of the basal diet were vitamin-test casein, salt mix (Wesson modification), and sucrose, purchased from General Biochemicals, Chagrin Falls, Ohio, and vitamin diet fortification mixture and cellulose, purchased from Nutritional Biochemicals, Cleveland, Ohio. Hydrogenated coconut oil and safflower oil were obtained from General Biochemicals and Nutritional Biochemicals, respectively. \([\text{U-}^{14}\text{C}]\)Glucose (specific activity, 14.7 mCi/mmmole), \([\text{1-}^{14}\text{C}]\)glucose (specific activity, 5.6 mCi/mmmole), \([\text{6-}^{14}\text{C}]\)glucose (specific activity, 4.85 mCi/mmmole), and \(^{14}\text{C}\)-labeled lipids, \([\text{1-}^{14}\text{C}]\)stearic acid, \([\text{1-}^{14}\text{C}]\)palmitic acid, \([\text{4-}^{14}\text{C}]\)cholesterol, and \([\text{1-}^{14}\text{C}]\)cholesterol palmitate, were from New England Nuclear Corp., Boston, Mass. Methyl esters of fatty acids (purity 95–99%) were obtained from Lipid Organic Research Elysian, Minn. Sephadex (G-25 coarse) was purchased by Pharmacia Fine Chemicals, Inc., Piscataway, N.J.; Unisol, from Clarkson Chemical Co., Williamport, Pa.; and Florisil, from Floridian Co., Pittsburgh, Pa. Squalene, tripalmitin, dipalmitin, diolein, monopalmitin, and monoolein were from Pierce Chemical Co., Rockford, Ill. Hyamine hydroxide was from Packard Instrument Co., Downers Grove, Ill. Gentamicin sulfate was purchased from Schering Corp., Bloomfield, N.J.; and PGE2 was a gift from Dr. John Pike of the Upjohn Co., Kalamazoo, Mich. Reagents were of analytical grade and solvents were redistilled before use.

**Induction of EFA deficiency in rats**

Male weanling Sprague-Dawley rats 15–20 days old and weighing 28–36 g were used in these experiments. Induction of EFA deficiency was according to the procedure described by Aaes-Jørgensen and Holman (16). The experimental group of animals was fed, ad lib., the basal diet supplemented with hydrogenated coconut oil; the diet of the control animals was supplemented with safflower oil. The animals were kept in individual cages in a room with controlled humidity and temperature (17). They were weighed and inspected for signs of deficiency at weekly intervals. The tail, forelegs, and hind legs were scored separately according to the dermal score described by Norby (18).

**Treatment of normal and EFA-deficient rats with PGE2**

For systemic administration PGE2 was dissolved in sterile 0.9% NaCl, and for each kilogram of body weight 0.1 ml of the solution containing 1 mg of PGE2 was injected intraperitoneally to the rat each day. For topical treatment, PGE2 was dissolved in a mixture of propylene glycol–ethanol 3:7 (v/v), and 0.1 ml of the solution containing 25, 50, or 100 μg of PGE2 was applied daily with a small brush to the dorsal or plantar surface of either a forelimb or hind limb. Care was taken that the solution was spread evenly without running. The ethanol was allowed to evaporate so that a thin film of the residual solution remained on the skin. The therapeutic effectiveness of the PGE2 was evaluated at weekly intervals according to the dermal score.

**Analysis of fatty acids in skin of control and EFA-deficient rats**

Skin specimens were removed from the shaved area of the posterior dorsum from normal and EFA-deficient rats as reported previously (19). The skin specimens were minced and homogenized in a mixture of chloroform–methanol 2:1 (20) with a Polytron (model PT 10) homogenizer. Tissue debris was removed by filtration on sintered glass funnels. The filtrate was evaporated in a rotary evaporator. The total lipids were transesterified by refluxing under nitrogen with 30 vol of a 5% solution of HCl in methanol. The methyl esters were analyzed by GLC, using an F & M model 402 with a hydrogen flame ionization detector. Glass columns, 182 cm × 3.5 mm, were packed with 3% diethylene glycol succinate polyester (DEGS) or 3% OV-101.
silicone copolymer coated on Supelcoport, 80–100 mesh (Supelco, Inc., Bellefonte, Pa.). When the DEGS column was used, the temperatures at the injection port, column, and detector were 190, 140, and 190°C, respectively; for the OV-101 column the temperatures were 210, 180, and 230°C, respectively. The nitrogen flow was 35 ml/min. The retention time was standardized by reference mixtures A, B, C, and D as specified by NIH. The methyl esters were identified by internal standards of reference methyl esters of fatty acids (purity 95–99%) obtained from the Hormel Institute. Quantification was by triangulation, and the fatty acid composition was expressed as percentages of the total fatty acids.

Preparation and incubation of skin specimens with differentially labeled glucose

Skin specimens from normal and EFA-deficient rats were prepared as described in a previous publication (19). Paired skin specimens (approximately 60–80 mg) were incubated in 2.0 ml of Krebs-Ringer bicarbonate solution (pH 7.4) gassed with 95% O₂ and 5% CO₂ and containing gentamicin sulfate (200 μg) and [U-¹⁴C]-glucose (5.0 μCi), [1-¹⁴C]glucose (2.5 μCi), or [6-¹⁴C]-glucose (2.5 μCi). PGE₂ (1 μg) was added to one of each pair of incubation flasks. For collection of ¹⁴CO₂, the method of Snyder and Godfrey (21) was modified: a polyethylene center well (Kontes Glass Co., Vineland, N.J.) was attached to a rubber cap (serum stopper) which was fitted to the top of a 10-ml Erlenmeyer flask containing the incubation mixture. After shaking at 37°C for 120 min, 0.3 ml of 1 M Hyamine hydroxide was introduced by injection through the rubber cap into the center well for trapping the CO₂, and 0.4 ml of 6 N H₂SO₄ was added to the incubation mixture to stop the reaction and to liberate ¹⁴CO₂. The vessel was left at room temperature for 4 hr, after which the center well was carefully removed and placed directly into a counting vial for radioassay.

Assay of ¹⁴C in lipid fractions

For the determination of ¹⁴C incorporated into lipids, after the incubation with ¹⁴C-labeled glucose, the skin specimen was rinsed gently with 0.9% saline and homogenized in a mixture of chloroform–methanol 2:1 with the Polytron homogenizer. Tissue debris was removed by filtration on a sintered glass funnel and was washed with the solvent mixture until no radioactivity could be detected in the wash. The filtrate was taken to dryness in a rotary evaporator. Nonlipid radioactive contaminant in the extract was removed by gel filtration on a column of Sephadex G-25 (coarse) as described by Siakotos and Rouser (22). The ¹⁴C in the column effluent was assayed by liquid scintillation counting. After evaporation of the solvents, the recovered lipid mixture was dissolved in chloroform and percolated onto a column containing 5 g of Unisil (100–200 mesh) suspended in chloroform, according to the procedure described by Borgström (23). Neutral lipids were eluted with chloroform (200 ml) and polar lipids with methanol (200 ml). Aliquots from each fraction were assayed for radioactivity.

The neutral lipids were dissolved in a small amount of hexane and were chromatographed further on 5 g of Florisil hydrated with 7% water and suspended in hexane, according to the procedure described by Carroll (24). Lipids were successively eluted from the column by the following solvents: (A) 15 ml of hexane; (B) 50 ml of 5% ether in hexane; (C) 75 ml of 15% ether in hexane; (D) 60 ml of 25% ether in hexane; (E) 40 ml of 50% ether in hexane; (F) 40 ml of 2% methanol in ether; and (G) 50 ml of 40% acetic acid in ether. The effluents were collected in 5-ml fractions, and aliquots were assayed for radioactivity. When reference compounds were chromatographed, squalene was eluted by (A); sterol and wax esters by (B); tripalmitin by (C); cholesterol by (D); dipalmitin and diolein by (E); monopalmitin and monoolein by (F), and palmitic acid and stearic acid by (G).

Thin-layer chromatography

For further resolution of fraction B, which usually contained both sterol esters and wax esters, thin-layer chromatography on silica gel G was performed according to the method of Schmid and Mangold (25), using the solvent system of hexane–diethyl ether 95:5 (v/v), with cholesteryl palmitate and stearyl palmitate as references. The ¹⁴C-labeled sterol esters were then eluted from the plates and saponified under nitrogen in 20% methanolic KOH at 80°C overnight in a closed vessel. The mixture was acidified with HCl and extracted with dichloromethane. The ¹⁴C was quantitatively recovered and the residue was subjected to thin-layer chromatography in the solvent system of isooctane–chloroform–acetic acid 20:4:1 (v/v/v) as described by Pocock, Marsden, and Hamilton (26), with cholesterol and palmitic acid as references.

RESULTS

Signs of EFA deficiency appeared after 6–8 wk, with increasing severity in scaliness in the skin as the experiment continued, in all animals fed the basal diet supplemented with hydrogenated coconut oil. The rats fed the diet containing safflower oil maintained a steady growth and did not exhibit any of the signs associated with deficiency of EFA.
TABLE 1. Fatty acid composition in skin of normal and EFA-deficient rats

<table>
<thead>
<tr>
<th>No. of Rats</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>20:3</th>
<th>20:4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>30.5</td>
<td>8.4</td>
<td>4.4</td>
<td>29.3</td>
<td>24.2</td>
<td>0.4</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.8</td>
<td>± 1.3</td>
<td>± 0.9</td>
<td>± 1.0</td>
<td>± 1.2</td>
<td>± 0.1</td>
<td>± 0.4</td>
</tr>
<tr>
<td>EFA-deficient</td>
<td>7</td>
<td>31.7</td>
<td>18.1</td>
<td>3.5</td>
<td>39.1</td>
<td>0.8</td>
<td>0.9</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 2.9</td>
<td>± 2.4</td>
<td>± 0.5</td>
<td>± 4.2</td>
<td>± 0.1</td>
<td>± 0.1</td>
<td>± 1.1</td>
</tr>
<tr>
<td>EFA-deficient (PGE2 treated)</td>
<td>2</td>
<td>35.7</td>
<td>14.8</td>
<td>7.4</td>
<td>36.6</td>
<td>1.1</td>
<td>0.8</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Standard mixtures of fatty acid methyl esters were obtained from the Hormel Institute.

TABLE 2. Effect of PGE2 on scaly lesions

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Rats</th>
<th>Treatment</th>
<th>Individual Dermal Score&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Average Score</th>
<th>Evaluation&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4</td>
<td>Saline, 0.1 ml, ip</td>
<td>0,0,0,0,0,0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>PGE2 (1 mg/kg/day) in 0.1 ml saline, ip</td>
<td>0,0,0,0,0,0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Vehicle, 0.1 ml, topical</td>
<td>0,0,0,0,0,0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>PGE2 in vehicle, 100 µg/day, topical</td>
<td>0,0,0,0,0,0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>EFA-deficient</td>
<td>4</td>
<td>Saline, 0.1 ml, ip</td>
<td>3,3,3,3,3,3</td>
<td>3</td>
<td>Ineffective</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>PGE2 (1 mg/kg/day) in 0.1 ml saline, ip</td>
<td>3,3,3,3,3,3</td>
<td>3</td>
<td>Ineffective</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Vehicle, 0.1 ml, topical</td>
<td>3,3,3,3,3,3</td>
<td>3</td>
<td>Ineffective</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>PGE2 in vehicle, 25 µg/day, topical</td>
<td>1,1,2,1,2,1</td>
<td>1.3</td>
<td>Slightly effective</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>PGE2 in vehicle, 50 µg/day, topical</td>
<td>0,1,0,0,1,1</td>
<td>0.5</td>
<td>Effective</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>PGE2 in vehicle, 100 µg/day, topical</td>
<td>0,0,0,0,0,0</td>
<td>0</td>
<td>Very effective</td>
</tr>
</tbody>
</table>

<sup>a</sup> 0, no scaling on the plantar or dorsum of feet; 1, slight scaling on the plantar and dorsum of feet; 2, moderate scaling on the plantar and dorsum of feet; 3, severe scaling on the plantar and dorsum of feet; —, not evaluated.

The fatty acid compositions of skin lipids of rats fed the two dietary regimens are given in Table 1. The changes in EFA deficiency included marked increases in monoenoic acids (16:1 and 18:1)<sup>1</sup> and in a fatty acid with chromatographic characteristics of eicosatrienoic acid (20:3), and decreases in dienoic (18:2) and tetranoic (20:4) acids. These changes in fatty acid composition in the skin were previously observed in other tissues in long-standing EFA deficiency (27-29). Topical application of PGE2, which caused the disappearance of the scaly lesions in the skin of EFA-deficient rats (see below), did not alter appreciably the profile of fatty acids in the skin (Table 1).

Effects of PGE2 on skin of normal and EFA-deficient rats

The rats fed safflower oil were divided into four groups of four animals each. Two groups were injected intraperitoneally with either 0.1 ml of sterile saline/kg body wt or 1 mg of PGE2 dissolved in 0.1 ml of saline/kg body wt (30). The other two groups were treated topically with 0.1 ml of a mixture of propylene glycol-ethanol 3:7 (v/v) or with PGE2 (100 µg/day) dissolved in the propylene glycol-ethanol mixture applied to the hind legs. Evaluation of the effects of PGE2 treatment is shown in Table 2. No ill effects were observed in the animals injected with PGE2 intraperitoneally. In all cases, no effects of PGE2, either systemic or topical, were discernible on the skin of the control rats.

The EFA-deficient rats were divided into six groups comprising four or six animals in each group. Two groups were injected with saline or a saline solution of PGE2 as described above, and no change in the cutaneous lesions was observed (Table 2). In some cases, physical conditions of the EFA-deficient rats worsened after the intraperitoneal injection of PGE2 and the animals died soon after the experimental period. The inability of PGE2 to clear the cutaneous scaly lesions when infused into EFA-deficient rats has been reported previously (30, 31). The lack of effect is probably due to rapid systemic metabolism of PGE2 to biologically inactive metabolites (32). The remaining four groups were treated topically either with a mixture of propylene glycol-ethanol 3:7 (v/v) or with three concentrations of PGE2 in the mixture, as shown in Table 2. These experiments demonstrated that the topical application of PGE2 at a concentration of 25 µg/day was slightly effective in clearing the scaly skin lesions of the EFA-deficient rats; 50

<sup>1</sup> Number of carbon atoms:number of double bonds.
μg was effective, and 100 μg was very effective. Representative photographs of the clearing effect are shown in Figs. 1 and 2.

In another experiment, each EFA-deficient rat was treated on one hind leg with PGE₂ dissolved in the vehicle and with the vehicle alone on the other. Thus, each animal served as its own control. The results showed that the scaly lesions cleared on the limb treated with PGE₂, but not on the limb treated with the vehicle (Fig. 2). These results demonstrated that the action of PGE₂ was localized in the area of application and did not exert its action on the other limbs via systemic circulation.

**Formation of CO₂ from [1-¹⁴C]glucose by skin of normal and EFA-deficient rats**

Previous studies in this laboratory demonstrated the formation of CO₂ from carbon 1 and carbon 6 of glucose by normal rat skin (33). For comparison, these experiments were again carried out with skin of EFA-deficient rats. The data in Fig. 3 show that the formation of CO₂ from glucose was enhanced in skin of EFA-deficient rats, and the enhancement was especially marked (almost threefold) in CO₂ formation from carbon 1.

**Lipid synthesis from [U-¹⁴C]glucose by skin of normal and EFA-deficient rats**

Results of the incorporation of uniformly labeled glucose in vitro into lipid fractions of normal and EFA-deficient rats are shown in Fig. 4. The incorporation of glucose carbon into neutral lipids was markedly increased in the EFA-deficient rats, while no significant change in the polar lipid fraction was observed.

In an attempt to ascertain whether the observed increase in the neutral lipids was in the synthesis of specific lipids, the neutral lipids from the normal and EFA-deficient rats were separated into seven fractions on the Florisil columns as described under Methods. The re-
Effects of PGE₂ on the synthesis of neutral lipids in normal and EFA-deficient rats

The marked increase in the synthesis of sterol esters in the skin of EFA-deficient rats was reminiscent of the observation of increased amounts of sterol esters in the adrenals of EFA-deficient rats (34, 35). The suggestion that increased sterol esterification might be related to abnormal keratinization in the skin (36, 37) spurred us to examine the effects of PGE₂ on the incorporation of ¹⁴C from glucose into this lipid fraction by skin of normal and EFA-deficient rats. Results of this experiment are shown in Fig. 6. The addition of PGE₂ to the incubation medium suppressed the incorporation of ¹⁴C from glucose into the sterol ester fraction in skin of normal and EFA-deficient rats. This inhibitory effect was par-
FIG. 5. Incorporation of [U-\(^{14}\)C]glucose into neutral lipids by skin of normal and EFA-deficient rats. The neutral lipids were separated on Florisil columns. Details of the method are described in the text. The data are from the averages of three experiments + SD.

A. Normal
- Control
- Control + PGE\(_2\)

B. EFA-Deficient
- EFA-Deficient
- EFA-Deficient + PGE\(_2\)

For the fraction containing squalene, it was interesting to observe that PGE\(_2\) exerted a minor but consistent stimulatory effect on the other neutral lipid fractions. This stimulation was consistent with our previous observation (15).

particularly pronounced (approximately 70%) in the skin of EFA-deficient rats. This finding was in harmony with recent reports describing the inhibition by PGE\(_1\) of cholesteryl ester synthetase in the liver (38), and of this enzyme by PGF\(_{2\alpha}\) in the corpus luteum (39). Except for the fraction containing squalene, it was interesting to observe that PGE\(_2\) exerted a minor but consistent stimulatory effect on the other neutral lipid fractions. This stimulation was consistent with our previous observation (15).
DISCUSSION

The changes in fatty acid composition of tissues previously described to occur in long-standing EFA deficiency (27-29) have now been observed in the skin. These changes, as shown in Table 1, included increases in monoenoic acids (16:1 and 18:1) and trienoic acid (20:3) and decreases in dienoic (18:2) and tetraenoic (20:4) acids. It seems that the increases in monoenoic and trienoic fatty acids under these conditions may be a compensatory response of the tissue to maintain a certain degree of total unsaturation of the tissue lipids that, under normal conditions, is provided by dietary EFA and their metabolites. The accumulation of eicosatrienoic acid (20:3) in the skin of EFA-deficient rats deserves attention, since Aaes-Jørgensen and Holman (16) considered its presence in a tissue a major criterion for the establishment of EFA-deficiency syndrome. Fulco and Mead (40) showed that this acid is derived from oleic acid (18:1) through a series of enzymatic transformations similar to those responsible for converting linoleic acid (18:2) into arachidonic acid (20:4). It may be speculated that in the absence or deficiency of linoleic acid indeed substitutes for arachidonic acid in the tissue the synthesis of this trienoic acid is accelerated.

The clearance by topical treatment with PGE2 of cutaneous scaly lesions induced in rats fed a diet deficient in EFA is of particular interest (Figs. 1 and 2). These results suggest possible connections between arachidonic acid, PGE2, and keratinization. The results further suggest that one of the functions of arachidonic acid in the skin may be to serve as the precursor for the production of PGE2, which in turn plays a role in regulating the normalcy of epidermal structures. Our results also showed that the action of PGE2 is limited to the local site and does not affect distant locales through systemic circulation (Fig. 2). This lack of systemic effect is further demonstrated by the intraperitoneal administration of PGE2 to EFA-deficient rats. Similar observations of the inability of PGE2 to clear cutaneous scaly lesions when infused into EFA-deficient rats have been reported previously (30, 31). The lack of effect is probably due to rapid systemic metabolism of PGE2 to biologically inactive metabolites (32).

Incubation of [1-14C]glucose with skin specimens from normal and EFA-deficient rats showed a marked increase in the oxidation of carbon 1 of glucose into 14CO2 by skin of EFA-deficient rats (Fig. 3). This result suggests an increased activity of the pentose cycle pathway which would generate increased amounts of NADPH necessary for lipogenesis. Consistent with this observation are the results from studies of lipid synthesis. Incubation of skin specimens from EFA-deficient rats with [U-14C]glucose showed marked increase in synthesis of lipids (Fig. 4), particularly the neutral lipids. Further resolution of the neutral lipids revealed a striking increase of synthesis in the sterol ester fraction with a moderate increase in the triglyceride fraction (Fig. 5). This finding is consistent with data reported by Sinclair (41), Kingery and Kellum (42), and Wilson (43), who found increased cholesteryl esters in the epidermis of rats fed a fat-free diet. Similar increases of cholesteryl esters in the adrenal tissue of EFA-deficient rats have also been reported by Walker (34) and Gidez (35).

It is noteworthy that the increased synthesis of sterol esters in the skin of the EFA-deficient rats occurs simultaneously with defects in the epidermal barrier system which are evidenced by the scaly lesions, the increased water consumption, and the diminished urine output by these animals. These data suggest a possible role of sterol esters in the barrier function. In man, the relationship of sterol esterification to keratinization has been suggested by reports of reduced ratios of esterified sterols to free sterols in psoriatic lesions (44) and scales (45). Although the biochemical events in the induction of scaly lesions in the skin of the EFA-deficient rats are not understood, it is tempting to speculate that the breakdown in the epidermal barrier in EFA-deficient rats resulting in increased transcutaneous permeability could be due to accumulation of abnormal sterol esters. Since the skin is known to contain other sterols in addition to cholesterol, the identification of the structures of the sterols as well as the sterol and the fatty acid moieties in the sterol esters remains for further studies. Phospholipids and cholesteryl esters normally contain polyunsaturated fatty acids and are constituents of cellular membranes. In EFA deficiency, cholesterol could be esterified with the monoenoic and trienoic fatty acids which are synthesized in greater amounts than normal. The appearance of these fatty acids in the cholesteryl ester fraction from the adrenals of EFA-deficient rats has already been reported (34, 35). Similarly, Mead and Slaton (46) have shown that the predominant fatty acid in the phospholipids of the heart and the liver of EFA-deficient animals is the abnormal 5,8,11-eicosatrienoic acid derived from oleic acid. De Tomás, Brenner, and Peluffo (47) reported that 5,8,11-eicosatrienoic acid indeed substitutes for arachidonic acid in the B position of these phospholipids. Sinclair (41) proposed that the esterification of abnormal fatty acids to cholesterol and phospholipids results in the accumulation of abnormal cholesteryl esters and phospholipids with deranged molecular spatial configurations. It is possible that the esterification of these fatty acids to sterols of the skin caused the breakdown in the membrane barrier of the skin of the EFA-deficient rats.

Results in Fig. 6 show that the presence of PGE2 in the incubation medium suppressed the synthesis of sterol esters in skin of both normal and EFA-deficient rats.
The inhibitory effect of PGE₂ was, however, more pronounced in the skin of the EFA-deficient rats. This difference could be due to the increased transcutaneous permeability in the EFA-deficient rats, thus permitting easier entry of PGE₂. Whether the clearance of the scaly lesions in the skin of the EFA-deficient rats is directly related to the inhibitory action of PGE₂ on the biosynthesis of sterol esters cannot be determined from the present data; nevertheless, the results do imply that PGE₂ plays a role in normalizing epidermal keratinization. The mode of action of PGE₂ in suppressing the synthesis of sterol esters was not clear, but two mechanisms could be possible. A direct inhibitory effect of PGE₂ on the skin cholesteryl ester synthesizing enzyme system is consistent with a recent report of the inhibition of hepatic cholesteryl ester synthetase by PGE₁ in vitro (38). Alternatively, there could be an activation of the sterol esterase (sterol ester hydrolase) in the skin by PGE₂, which would result in a decrease of the accumulated sterol esters. The latter possibility must await the demonstration of a sterol ester hydrolase in the skin.

The skin has thus proved a particularly useful tissue for the evaluation of the biological importance of EFA. Because the development of the cutaneous lesions of EFA deficiency can be observed visually and treated topically and sequential biopsy specimens can be obtained without killing the animal, this organ offers some distinct advantages as an experimental model.

The authors wish to express their thanks to Mrs. Carmen Marquez and Mr. Rudy Guerra for their valuable technical assistance during the course of this study.

This investigation was supported in part by grant HE 14141 from the National Heart and Lung Institute of the U.S. Public Health Service and a research grant from Hoffmann-La Roche, Inc., Nutley, N.J.

Manuscript received 22 November 1971; accepted 6 March 1972.

REFERENCES


