Effects of prostaglandin E₂ on rat skin: inhibition of sterol ester biosynthesis and clearing of scaly lesions in essential fatty acid deficiency

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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 monoenoic 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 E₂ (PGE₂) 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 PGE₂ had no observable effect on the skin lesions. Furthermore, incubation of skin specimens from the EFA-deficient rats with ¹⁴C-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 PGE₂ 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 PGE₂ 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

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 oleate, no increase in polyunsaturated acids in these organs could be detected. Greenberg et al. (10) reported that methyl arachidonate pos-

T HE 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

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 PGE_2 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 PGE_2 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 PGE_2 and that PGE_2 exerts a curative effect on skin lesions in EFA deficiency suggest that the biochemical action of PGE_2 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 PGE_2 markedly inhibits the biosynthesis of sterol esters by skin of the EFA-deficient rats. It is tempting to associate the curative effects of PGE_2 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 PGE_2 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. [U-^{14}C]Glucose (specific activity, 14.7 mCi/mmole), [1-^{14}C]glucose (specific activity, 5.6 mCi/mmole), [6-^{14}C]glucose (specific activity, 4.85 mCi/mmole), and ^{14}C-labeled lipids, [1-^{14}C]stearic acid, [1-^{14}C]palmitic acid, [4-^{14}C]cholesterol, and [1-^{14}C]-cholesteryl 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 from Pharmacia Fine Chemicals, Inc., Piscataway, N.J.; Unisil, from Clarkson Chemical Co., Williamsport, Pa.; and Florisil, from Floridin 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 PGE_2 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 Nørby (18).

Treatment of normal and EFA-deficient rats with PGE_2

For systemic administration PGE_2 was dissolved in sterile 0.9% NaCl, and for each kilogram of body weight 0.1 ml of the solution containing 1 mg of PGE_2 was injected intraperitoneally to the rat each day. For topical treatment, PGE_2 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 PGE_2 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 PGE_2 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% O_2 and 5% CO_2 and containing gentamicin sulfate (200 μg) and [U-^{14}C]-glucose (5.0 μCi), [1-^{14}C]glucose (2.5 μCi), or [6-^{14}C]-glucose (2.5 μCi). PGE$_2$ (1 μg) was added to one of each pair of incubation flasks. For collection of $^{14}CO_2$, 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 CO_2, and 0.4 ml of 6 N H_2SO_4 was added to the incubation mixture to stop the reaction and to liberate $^{14}CO_2$. 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 ^{14}C in lipid fractions

For the determination of ^{14}C incorporated into lipids, after the incubation with ^{14}C-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 ^{14}C in the column effluent was assayed by liquid scintillation counting. After evap-

oration 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 ^{14}C-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 ^{14}C 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

	No. of Rats	16:0	16:1	18:0	18:1	18:2	18:3	20:3	20:4
						% of total fatty acids			
Control	4	30.5 ± 0.8	8.4 ± 1.3	4.4 ± 0.9	29.3 ± 1.0	24.2 ± 1.2	trace	0.4 ± 0.1	2.4 ± 0.4
EFA-deficient	7	31.7 ± 2.9	18.1 ± 2.4	3.5 ± 0.5	39.1 ± 4.2	0.8 ± 0.1	0.9 ± 0.1	5.2 ± 1.1	0.9 ± 0.1
EFA-deficient (PGE$_2$ treated)	2	35.7	14.8	7.4	36.6	1.1	0.8	3.7	0.8

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

TABLE 2. Effect of PGE$_2$ on scaly lesions

Condition	No. of Rats	Treatment	Individual Dermal Score[a]	Average Score	Evaluation[b]
Normal	4	Saline, 0.1 ml, ip	0,0,0,0,—,—	0	—
	4	PGE$_2$ (1 mg/kg/day) in 0.1 ml saline, ip	0,0,0,0,—,—	0	—
	4	Vehicle,[c] 0.1 ml, topical	0,0,0,0,—,—	0	—
	4	PGE$_2$ in vehicle, 100 μg/day, topical	0,0,0,0,—,—	0	—
EFA-deficient	4	Saline, 0.1 ml, ip	3,3,3,3,—,—	3	Ineffective
	4	PGE$_2$ (1 mg/kg/day) in 0.1 ml saline, ip	3,3,3,3,—,—	3	Ineffective
	6	Vehicle, 0.1 ml, topical	3,3,3,3,3,3	3	Ineffective
	6	PGE$_2$ in vehicle, 25 μg/day, topical	1,1,2,1,2,1	1.3	Slightly effective
	6	PGE$_2$ in vehicle, 50 μg/day, topical	0,1,0,0,1,1	0.5	Effective
	6	PGE$_2$ in vehicle, 100 μg/day, topical	0,0,0,0,0,0	0	Very effective

[a] 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.

[b] The effectiveness of PGE$_2$ was evaluated after 14 days of treatment. Average score of 1 or less was considered as effective in clearing the scaly lesions. Details of experimental procedure are described under Methods.

[c] The vehicle was a mixture of propylene glycol–ethanol 3:7 (v/v) used for the topical application of PGE$_2$.

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)[1] and in a fatty acid with chromatographic characteristics of eicosatrienoic acid (20:3), and decreases in dienoic (18:2) and tetraenoic (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 PGE$_2$, 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 PGE$_2$ 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 PGE$_2$ 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 PGE$_2$ (100 μg/day) dissolved in the

propylene glycol–ethanol mixture applied to the hind legs. Evaluation of the effects of PGE$_2$ treatment is shown in Table 2. No ill effects were observed in the animals injected with PGE$_2$ intraperitoneally. In all cases, no effects of PGE$_2$, 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 PGE$_2$ 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 PGE$_2$ and the animals died soon after the experimental period. The inability of PGE$_1$ 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 PGE$_2$ 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 PGE$_2$ in the mixture, as shown in Table 2. These experiments demonstrated that the topical application of PGE$_2$ at a concentration of 25 μg/day was slightly effective in clearing the scaly skin lesions of the EFA-deficient rats; 50

[1] 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 PGE_2 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 PGE_2, but not on the limb treated with the vehicle (Fig. 2). These results demonstrated that the action of PGE_2 was localized in the area of application and did not exert its action on the other limbs via systemic circulation.

Formation of CO_2 from $[1\text{-}^{14}C]$glucose by skin of normal and EFA-deficient rats

Previous studies in this laboratory demonstrated the formation of CO_2 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 CO_2 from glucose was enhanced in skin of EFA-deficient rats, and the enhancement was especially marked (almost threefold) in CO_2 formation from carbon 1.

Lipid synthesis from $[U\text{-}^{14}C]$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-
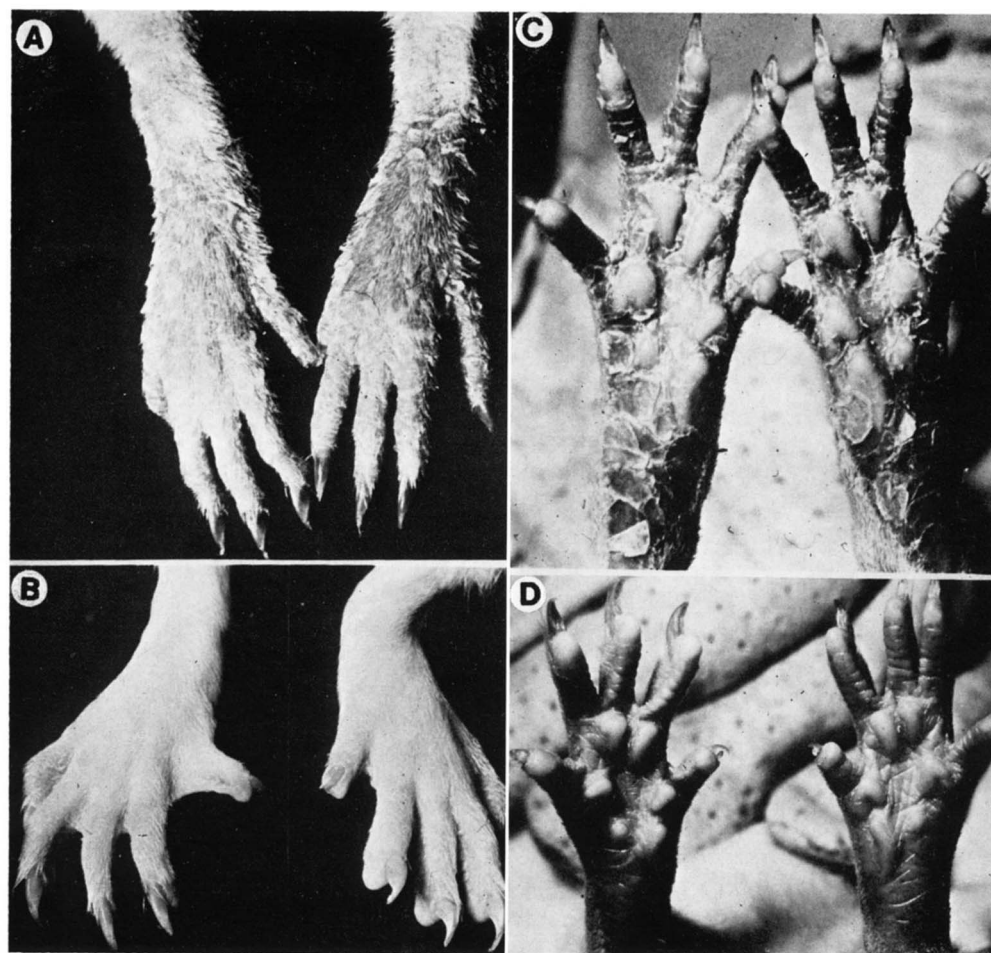

FIG. 1. Effect of PGE_2 on scaly lesions of EFA-deficient rats. *A*, severe scaling on the dorsum of the hind limbs of an EFA-deficient rat remains after 2 wk of topical treatment with propylene glycol–ethanol 3:7 (v/v). *B*, clearance of the lesions after 2 wk of topical treatment with PGE_2 dissolved in propylene glycol–ethanol 3:7 (v/v). *C*, severe scaling on the plantar surface of the hind limbs of an EFA-deficient rat remains after 2 wk of topical treatment with propylene glycol–ethanol 3:7 (v/v). *D*, clearance of the scaly lesions on the plantar surface of the hind limbs of an EFA-deficient rat after 2 wk of topical treatment with PGE_2 dissolved in propylene glycol–ethanol 3:7 (v/v).
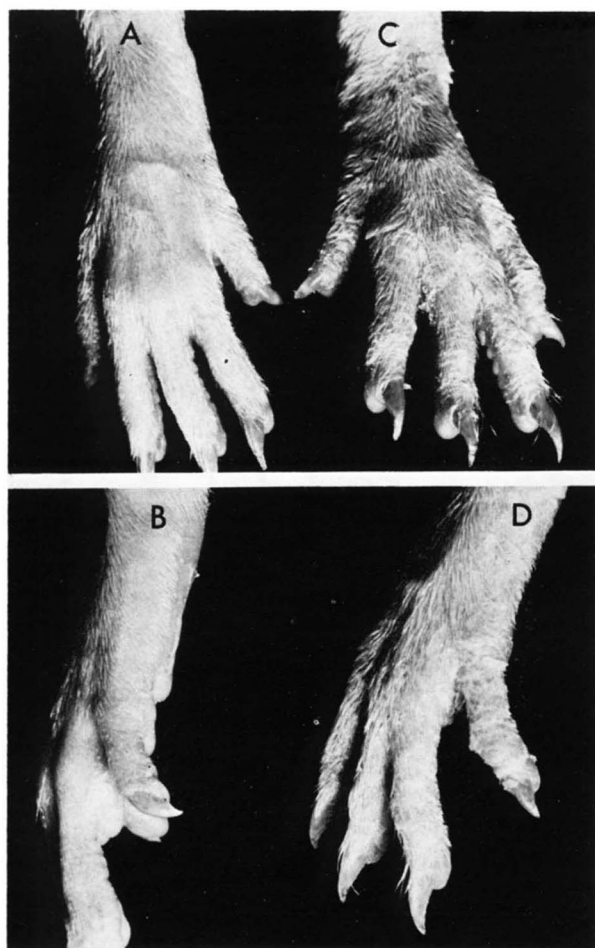
Fig. 2.   Application of PGE₂ and vehicle to scaly hind limbs of the same rat. *A* and *B*, clearance of scaly lesions on the dorsum of one hind leg of an EFA-deficient rat after 2 wk of topical treatment with PGE₃ dissolved in the vehicle. *C* and *D*, no change in scaly lesions on the dorsum of the other hind leg of an EFA-deficient rat after 2 wk of topical treatment with the vehicle.
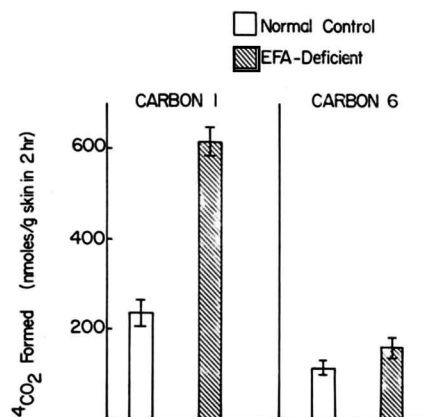

Fig. 3.   Oxidation of [1-¹⁴C]glucose and [6-¹⁴C]glucose to ¹⁴CO₂ by skin of normal and EFA-deficient rats. Methods of incubation and ¹⁴CO₂ determination are described in the text. The data are from the averages of three experiments ± SD.

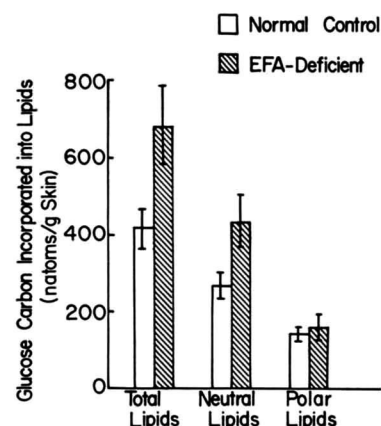Fig. 4.   Incorporation of [U-¹⁴C]glucose into lipids by skin of normal and EFA-deficient rats. Methods of incubation and recovery of lipids are as described in the text. The number of nano-atoms of glucose carbon was calculated by dividing the amount of ¹⁴C in the lipids by the specific activity of [U-¹⁴C]glucose in the medium and multiplying by 6. The data are the averages of three experiments ± SD.

sults are shown in Fig. 5. The striking difference in the neutral lipids from these two groups of animals is the 4–5-fold increase of ¹⁴C incorporated into the sterol and wax ester fraction of the EFA-deficient rats. This fraction was further subjected to thin-layer chromatography to separate the sterol esters from the wax esters, as described by Schmid and Mangold (25). Over 94% of the ¹⁴C in this fraction had a chromatographic mobility corresponding to a reference of cholesteryl palmitate. Less than 1% of the ¹⁴C could be accounted for in the spot corresponding to the mobility of stearyl palmitate. After hydrolysis of the sterol ester fraction recovered from the thin-layer plates, the resulting products were subjected to thin-layer chromatography in the system described by Pocock et al. (26). The ¹⁴C was quantitatively recovered in the zones of sterols (68%, cholesterol as reference) and fatty acids (28%, palmitic acid as reference).

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-

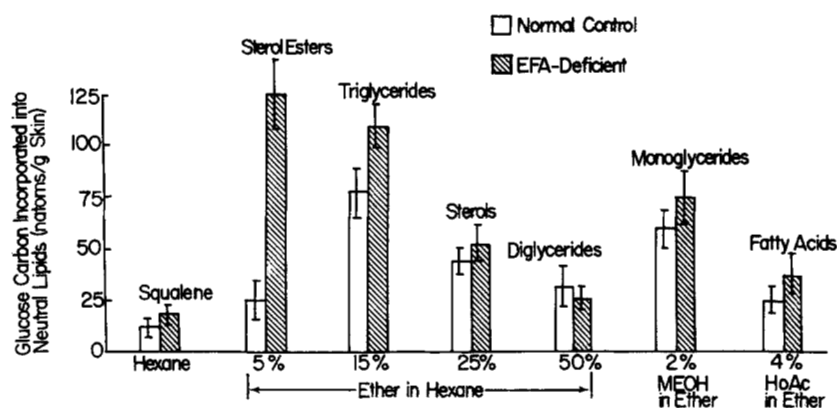FIG. 5. Incorporation of [U-¹⁴C]glucose into neutral lipids by skin of normal and EFA-deficient rats. The neutral lipids were separated on Florisil columns. Details of the method are described in the text. The data are from the averages of three experiments ± SD.
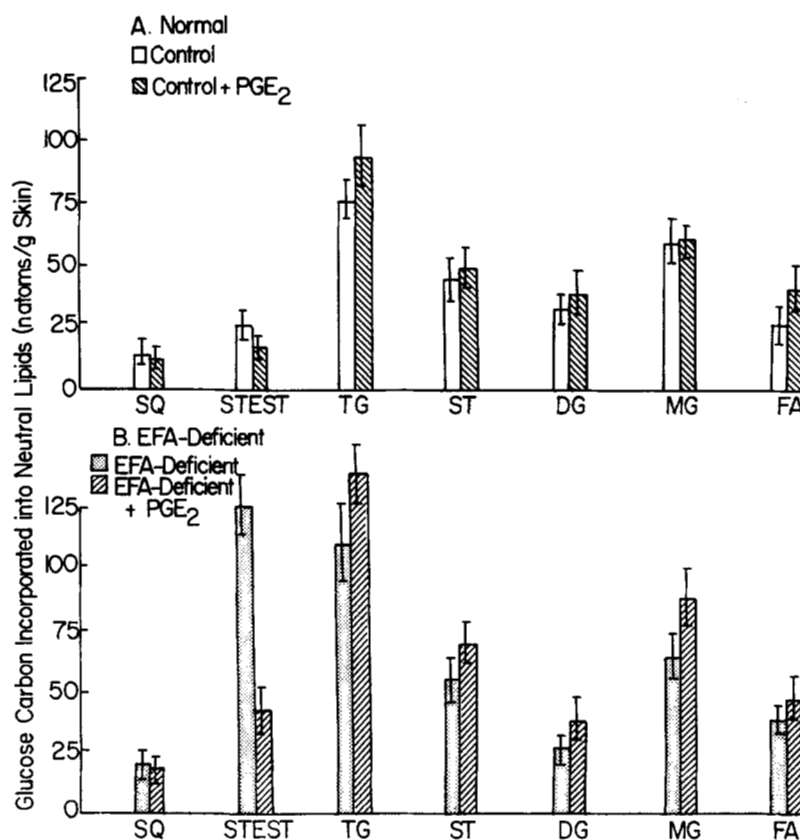

FIG. 6. Inhibition of sterol ester synthesis of PGE₂. PGE₂ (0.5 µg/ml) was added to the incubation mixture consisting of Krebs-Ringer bicarbonate buffer, pH 7.4, antibiotic, skin from normal (A) or EFA-deficient rats (B), and [U-¹⁴C]glucose (5 µCi, 0.36 µmole). The amounts of ¹⁴C incorporated from radioactive glucose into neutral lipid fractions were determined by the procedures described in the text. The data were obtained from the averages of three experiments ± SD. SQ, squalene; STEST, sterol and wax esters; TG, triglycerides; ST, sterols; DG, diglycerides; MG, monoglycerides; and FA, fatty acids.

ticularly pronounced (approximately 70%) in the skin of EFA-deficient rats. This finding was in harmony with recent reports describing the inhibition by PGE₁ of cholesteryl ester synthetase in the liver (38), and of this enzyme by PGF₂α in the corpus luteum (39). Except

for the fraction containing squalene, it was interesting to observe that PGE₂ 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 d'etary 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 in the tissue the synthesis of this trienoic acid is accelerated.

The clearance by topical treatment with PGE_2 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, PGE_2, 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 PGE_2, which in turn plays a role in regulating the normalcy of epidermal structures. Our results also showed that the action of PGE_2 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 PGE_2 to EFA-deficient rats. Similar observations of the inability of PGE_1 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 PGE_2 to biologically inactive metabolites (32).

Incubation of [1-^{14}C]glucose with skin specimens from normal and EFA-deficient rats showed a marked increase in the oxidation of carbon 1 of glucose into $^{14}CO_2$ 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-^{14}C]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 β 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 PGE_2 in the incubation medium suppressed the synthesis of sterol esters in skin of both normal and EFA-deficient rats.

The inhibitory effect of PGE_2 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 PGE_2. Whether the clearance of the scaly lesions in the skin of the EFA-deficient rats is directly related to the inhibitory action of PGE_2 on the biosynthesis of sterol esters cannot be determined from the present data; nevertheless, the results do imply that PGE_2 plays a role in normalizing epidermal keratinization. The mode of action of PGE_2 in suppressing the synthesis of sterol esters was not clear, but two mechanisms could be possible. A direct inhibitory effect of PGE_2 on the skin cholesteryl ester synthesizing enzyme system is consistent with a recent report of the inhibition of hepatic cholesterol ester synthetase by PGE_1 in vitro (38). Alternatively, there could be an activation of the sterol esterase (sterol ester hydrolase) in the skin by PGE_2, 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.

REFERENCES

1. Burr, G. O., and M. M. Burr. 1929. A new deficiency disease produced by rigid exclusion of fat from the diet. *J. Biol. Chem.* **82:** 345–367.
2. Burr, G. O., and M. M. Burr. 1930. On nature and role of the fatty acids essential in nutrition. *J. Biol. Chem.* **86:** 587–621.
3. Smedley-MacLean, I., and L. C. A. Nunn. 1940. Fat-deficiency disease of rats. The effect of doses of methyl arachidonate and linoleate on fat metabolism, with a note on the estimation of arachidonic acid. *Biochem. J.* **34:** 884–902.
4. Turpeinen, O. 1938. Further studies on the unsaturated fatty acids essential in nutrition. *J. Nutr.* **15:** 351–366.
5. Barki, V. H., R. A. Collins, E. B. Hart, and C. A. Elvehjem. 1949. Relation of fatty deficiency symptoms to the polyunsaturated fatty acid content of the tissues of the mature rat. *Proc. Soc. Exp. Biol. Med.* **71:** 694–696.
6. Reiser, R. 1950. The metabolism of polyunsaturated fatty acids in growing chicks. *J. Nutr.* **42:** 325–336.
7. Reiser, R. 1951. The syntheses and interconversions of polyunsaturated fatty acids by the laying hen. *J. Nutr.* **44:** 159–175.
8. Rieckehoff, I. G., R. T. Holman, and G. O. Burr. 1949. Polyethenoid fatty acid metabolism. Effect of dietary fat on polyethenoic fatty acids of rat tissues. *Arch. Biochem.* **20:** 331–340.
9. Widmer, C., Jr., and R. T. Holman. 1950. Polyethenoic fatty acid metabolism. II. Deposition of polyunsaturated fatty acids in fat-deficient rats upon single fatty acid supplementation. *Arch. Biochem. Biophys.* **25:** 1–12.
10. Greenberg, S. M., C. E. Calbert, H. J. Deuel, Jr., and J. B. Brown. 1951. The effect of fat level of the diet on general nutrition. VII. Comparison of the potency of arachidonic and linoleic acids in furnishing the requirement for essential fatty acids in the rat. *J. Nutr.* **45:** 521–533.
11. Van Dorp, D. A., R. K. Beerthuis, D. H. Nugteren, and H. Von Keman. 1964. The biosynthesis of prostaglandins. *Biochim. Biophys. Acta.* **90:** 204–207.
12. Bergström, S., H. Danielsson, and B. Samuelsson. 1964. The enzymatic formation of prostaglandin E_2 from arachidonic acid. Prostaglandins and related factors 32. *Biochim. Biophys. Acta.* **90:** 207–210.
13. Van Dorp, D. A., R. K. Beerthuis, D. H. Nugteren, and H. Von Keman. 1964. Enzymatic conversion of all-*cis*-polyunsaturated fatty acids into prostaglandins. *Nature.* **203:** 839–841.
14. Christ, E. J., and D. H. Nugteren. 1970. The biosynthesis and possible function of prostaglandins in adipose tissue. *Biochim. Biophys. Acta.* **218:** 296–307.
15. Ziboh, V. A., and S. L. Hsia. 1971. Prostaglandin E_2: biosynthesis and effects on glucose and lipid metabolism in rat skin. *Arch. Biochem. Biophys.* **146:** 100–109.
16. Aaes-Jørgensen, E., and R. T. Holman. 1958. Essential fatty acid deficiency. I. Content of polyenoic acids in testes and heart as an indicator of EFA status. *J. Nutr.* **65:** 633–641.
17. Aaes-Jørgensen, E. 1961. Essential fatty acids. *Physiol. Rev.* **41:** 1–51.
18. Nørby, J. G. 1965. Effects of giving fat-free diet for up to 10 weeks on the male weanling rat. *Brit. J. Nutr.* **19:** 209–224.
19. Ziboh, V. A., M. A. Dreize, and S. L. Hsia. 1970. Inhibition of lipid synthesis and glucose-6-phosphate dehydrogenase in rat skin by dehydroepiandrosterone. *J. Lipid Res.* **11:** 346–354.
20. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
21. Snyder, F., P. Godfrey. 1961. Collecting $C^{14}O_2$ in a Warburg flask for subsequent scintillation counting. *J. Lipid Res.* **2:** 195.
22. Siakotos, A. N., and G. Rouser. 1965. Analytical separation of nonlipid water soluble substances and gangliosides from other lipids by dextran gel column chromatography. *J. Amer. Oil Chem. Soc.* **42:** 913–919.
23. Borgström, B. 1952. Investigation on lipid separation methods. Separation of phospholipids from neutral fat and fatty acids. *Acta Physiol. Scand.* **25:** 101–110.
24. Carroll, K. K. 1961. Separation of lipid classes by chromatography on Florisil. *J. Lipid Res.* **2:** 135–141.
25. Schmid, H. H. O., and H. K. Mangold. 1966. Neutrale Plasmalogene und Alkoxy-diglyceride im menschlichen Depotfett. *Biochem. Z.* **346:** 13–25.

26. Pocock, D. M. E., J. R. Marsden, and J. G. Hamilton. 1969. The presence of alkenyl and alkyl glycerides, and choline and ethanolamine plasmalogens in a marine worm. *Comp. Biochem. Physiol.* **30:** 133–136.

27. Morton, R. A., and A. A. Horner. 1961. The liver-lipid constituents of male and female rats. I. Effects of the fat deficiency syndrome. *Biochem. J.* **79:** 631–635.

28. Mohrhauer, H., and R. T. Holman. 1963. The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver. *J. Lipid Res.* **4:** 151–159.

29. Mohrhauer, H., and R. T. Holman. 1963. The effect of dietary essential fatty acids upon composition of polyunsaturated fatty acids in depot fat and erythrocytes of the rat. *J. Lipid Res.* **4:** 346–350.

30. Kupiecki, F. P., N. C. Sekhar, and J. R. Weeks. 1968. Effects of infusion of some prostaglandins in essential fatty acid-deficient and normal rats. *J. Lipid Res.* **9:** 602–605.

31. Gottenbos, J. J., R. K. Beerthuis, and D. A. Van Dorp. 1967. Essential fatty acid bioassay of prostaglandin E$_1$ in rats and mice. *In* Nobel Symposium 2: Prostaglandins. S. Bergstrom and B. Samuelsson, editors. Almquist and Wiksell, Stockholm; Interscience, New York. 57–62.

32. Ferreira, S. H., and J. R. Vane. 1967. Prostaglandins: their disappearance from and release into the circulation. *Nature.* **216:** 868–873.

33. Ziboh, V. A., R. Wright, and S. L. Hsia. 1971. Effects of insulin on the uptake and metabolism of glucose by rat skin in vitro. *Arch. Biochem. Biophys.* **146:** 93–99.

34. Walker, B. L. 1970. The fatty acids of adrenal lipids from essential fatty acid-deficient rats. *J. Nutr.* **100:** 355–360.

35. Gidez, L. I. 1964. Occurrence of a docosatrienoic acid in the cholesterol esters of adrenals of rats on essential fatty acid deficient diets. *Biochem. Biophys. Res. Commun.* **14:** 413–418.

36. Unna, P. G., and L. Golodetz. 1910. Die Cholesterinester der Hornschicht. *Biochem. Z.* **25:** 425–527.

37. Kooyman, D. J. 1932. Lipids of the skin. Some changes in the lipids of the epidermis during the process of keratinization. *Arch. Dermatol.* **25:** 444–450.

38. Schweppe, J. S., and R. A. Jungmann. 1970. Prostaglandins: the inhibition of hepatic cholesterol ester synthesase in the rat. *Proc. Soc. Exp. Biol. Med.* **133:** 1307–1309.

39. Behrman, H. R., G. J. MacDonald, and R. O. Greep. 1971. Regulation of ovarian cholesterol esters: evidence for the enzymatic sites of prostaglandin-induced loss of corpus luteum function. *Lipids.* **6:** 791–796.

40. Fulco, A. J., and J. F. Mead. 1959. Metabolism of essential fatty acids. VIII. Origin of 5,8,11-eicosatrienoic acid in the fat-deficient rat. *J. Biol. Chem.* **234:** 1411–1416.

41. Sinclair, H. M. 1956. Deficiency of essential fatty acids in relation to coronary thrombosis. Third International Conference on Biochemical Problems of Lipids, Brussels. 392–396.

42. Kingery, F. A. J., and R. E. Kellum. 1965. Essential fatty acids. Histochemical changes in the skin of rats. *Arch. Dermatol.* **91:** 272–279.

43. Wilson, J. D. 1963. Studies on the regulation of cholesterol synthesis in the skin and preputial gland of the rat. *Advan. Biol. Skin.* **4:** 148–166.

44. Marchionini, A., E. Manz, and F. Huss. 1938. Der Cholesteringehalt der Hautsberschicht bei der Seborrhoe und bei der Psoriasis. *Arch. Derm. Syph.* **176:** 613.

45. Reinertson, R. P., and V. R. Wheatley. 1959. Studies on the chemical composition of human epidermal lipids. *J. Invest. Dermatol.* **32:** 49–59.

46. Mead, J. F., and W. H. Slaton, Jr. 1956. Metabolism of essential fatty acids. III. Isolation of 5,8,11-eicosatrienoic acid from fat-deficient rats. *J. Biol. Chem.* **219:** 705–709.

47. De Tomás, M. E., R. R. Brenner, and R. O. Peluffo. 1933. Position of eicosatrienoic acid in phosphatidylcholine and phosphatidylethanolamine from rats deficient in essential fatty acids. *Biochim. Biophys. Acta.* **70:** 472–474.