Metabolism of essential fatty acids by human epidermal enzyme preparations: evidence of chain elongation[1]

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Abstract The present studies were undertaken in order to delineate the source of human epidermal arachidonic acid, 20:4(n-6). Epidermal microsomal preparations from normal (N) and diseased epidermis (clinically uninvolved (PU) and involved psoriatic (PI) epidermis) were incubated in vitro with either [^{14}C]18:2(n-6), [^{14}C]20:3(n-6) or [^{14}C]malonyl CoA to determine the activities of the Δ^6, Δ^5 desaturases and elongase, respectively. Experiments were performed in parallel with rat liver microsomal preparations where enzyme activities are well documented. Data derived from the enzymatic assays were compared to fatty acid composition data derived from epidermal total lipids. The enzymatic conversion rates were determined after methylation and separation of the ^{14}C-labeled fatty acid methyl esters by argentation thin-layer chromatography and reverse phase high-performance liquid chromatography. GLC Our data demonstrated: *i)* that N, PU, and PI epidermis were all capable of elongating 18:3(n-6) into ^{14}C-labeled 20:3(n-6) via the addition of [^{14}C]malonyl CoA, and this activity was markedly elevated (fivefold) in PI preparations; *ii)* that N, PU, and PI epidermal preparations lacked the capacity to desaturate 18:2(n-6) and 20:3(n-6); and *iii)* striking alterations in the individual fatty acids (as weight percent) in the total fatty acids of the PI epidermal extracts when compared to the PU and N extracts. These findings indicate that epidermal arachidonic acid is not biosynthesized locally from tissue linoleic acid and must, therefore, depend on contribution from another endogenous source. — **Chapkin, R. S., V. A. Ziboh, C. L. Marcelo, and J. J. Voorhees.** Metabolism of essential fatty acids by human epidermal enzyme preparations: evidence of chain elongation. *J. Lipid Res.* 1986. **27:** 945-954.

Supplementary key words desaturation • elongation • psoriasis • epidermis

The importance of unsaturated fatty acids, especially linoleic (18:2(n-6)) and arachidonic acid (20:4(n-6)) in normal mammalian physiological function was first established by Burr and Burr in 1929 (1). This recognition, that some unsaturated fatty acids which cannot be synthesized by mammalian tissues are 'essential' dietary elements, led to the designation of these acids as either essential or nonessential. It is also well recognized that the essential fatty acids (EFA) serve a structural function as an integral part of phospholipids (one of the components of all biomembranes).

The skin, predominantly its epidermal layer, is an organ displaying a highly active metabolism of 20:4(n-6). The level and replenishment of this fatty acid in the epidermis is vital for normal epidermal function. Metabolites of arachidonic acid in skin have been associated with inflammation, growth regulation, and differentiation in this tissue. For instance, increases in the concentrations of 20:4(n-6), certain prostaglandins, and leukotrienes in skin are associated with many inflammatory dermatoses (2). Cyclooxygenase and lipoxygenase products of 20:4(n-6), acting either alone or in synergism with other compounds, have been implicated in the pathogenesis of psoriasis vulgaris, a multifactorial chronic inflammatory skin disease (3, 4).

In many animal tissues and cells, 18:2(n-6) is converted to 20:4(n-6) by an alternating sequence of Δ^6 desaturation, chain elongation, and Δ^5 desaturation (5). The enzymes that catalyze these biochemical events are localized in the endoplasmic reticulum of many tissues (5). It is unclear, however, whether 20:4(n-6), which is found in skin phospholipids, originates from the liver or whether it

Abbreviations: N, normal; PU, uninvolved psoriatic; PI, involved psoriatic; GLC, gas-liquid chromatography; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; GSH, reduced glutathione; EFA, essential fatty acid; PUFA, polyunsaturated fatty acids; PGE_1 prostaglandin E_1 (11,15-dihydroxy-9-oxoprosta-13-enoic acid); CoA, coenzyme A; TEWL, transepidermal water loss. Fatty acids are designated by number of carbon atoms:number of double bonds; "n" indicates the number of carbon atoms from the methyl end of the acyl chain to the nearest double bond.

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is biosynthesized locally in the epidermis. We have previously demonstrated (6) that epidermal enzyme preparations from rat and guinea pig skin lack the capacity to transform 18:2(n-6) and 20:3(n-6) (dihomo-γ-linolenic acid) into 20:4(n-6), implying that the epidermal 20:4(n-6) in the skin of these animals is not biosynthesized locally by the skin but possibly transported to the epidermis for esterification into the phospholipids. However, the origin of 20:4(n-6) in human epidermis has not been examined. Because of the recognition that arachidonic acid is an essential constituent of human epidermal phospholipids and the precursor of the variety of eicosanoids in this tissue, coupled with the increasing use of primrose oil (containing 18:3(n-6)) in the management of human cutaneous disorders, we were prompted to investigate *i)* whether enzyme preparations from normal (N) as well as clinically involved (PI) and uninvolved (PU) psoriatic epidermis have the capacity to desaturate linoleic acid (18:2(n-6)) to γ-linolenic acid (18:3(n-6)) as well as 20:3(n-6) to arachidonic acid (20:4(n-6)); and *ii)* whether these preparations also have the capacity to elongate 18:3(n-6) into the 20:3(n-6) acid. The activities of the three enzymes involved in these transformations were determined in vitro using microsomal and cytosolic preparations from human skin epidermis performed in parallel with microsomal preparations from rat liver, where enzyme activities are well documented (7). This study also compares the analysis of the fatty acid composition of total lipids in the epidermis by a discriminating gas-liquid chromatography (GLC) method with data derived from enzymatic assays.

MATERIALS AND METHODS

Materials

[1-^{14}C]Linoleic acid (18:2(n-6)) (57 mCi/mmol, 98% radiochemical purity) was purchased from Amersham (Arlington Heights, IL). [1-^{14}C]Dihomo-γ-linolenic acid (20:3(n-6)) (54.9 mCi/mmol, 99% radiochemical purity) and [2-^{14}C]malonyl CoA (50.5 mCi/mmol, 99% radiochemical purity) were purchased from New England Nuclear (Boston, MA). NADH and coenzyme A (CoA) were purchased from Calbiochem-Behring (San Diego, CA); reduced glutathione (GSH), ATP, and NADPH were purchased from Boehringer Mannheim (Indianapolis, IN). Malonyl CoA (lithium salt) was obtained from Sigma Chemical Co. (St. Louis, MO). All unlabeled fatty acids and methyl esters were purchased from Nu-Chek-Prep (Elysian, MN) and were checked for purity. Sephadex G-25 (coarse) was purchased from Pharmacia Fine Chemicals, Inc. (Piscataway, NJ). Silica gel G was obtained from E. Merck Co. (Darmstadt, West Germany). Adam's catalyst (PtO_2) was purchased from Aldrich Chemical Co. (Milwaukee, WI). All chemicals and solvents were of analytical grade or high performance liquid chromatography (HPLC) quality. HPLC quality solvents were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Aqueous and HPLC quality solvents were filtered through 0.45 μm and 5 μm filters (Millipore Co., Bedford, MA), respectively, and degassed prior to use.

Skin biopsies

Whole epidermal biopsies of 0.4-mm thickness were taken from clinically involved areas of skin from 12 psoriatic subjects with a Castroviejo keratome blade (Storz Co., St. Louis, MO) after obtaining a signed informed consent form, which had been previously approved by the University of Michigan Committee for Protection of Human Research. Specimens (0.2 mm) were taken from uninvolved areas of the skin from the same psoriatic subjects. For controls, whole epidermal biopsy specimens of 0.2-mm thickness were taken from each of 10 normal volunteers. Representative sections from the specimens were taken for histologic estimation of thickness and the remaining portions were snap-frozen in liquid nitrogen and stored at −70°C until homogenized and used for incubations.

Preparation of high speed microsomal fractions

Individual keratome slices were divided into two separate pools and placed in an ice-cold homogenizing medium (0.25 M sucrose solution containing 0.15 M KCl, 5 mM $MgCl_2$, 1.5 mM GSH, and 1 mM EDTA, adjusted to pH 7.0 with 0.05 M potassium phosphate buffer) and homogenized at 4°C with a Polytron homogenizer (Model PT 10, Brinkmann Instruments, Westbury, NY). The microsomal fractions were prepared as reported previously by Ziboh, Dreize, and Hsia (8). The 105,000 *g* supernatant fluid was concentrated at 0°C to one quarter its volume with Sephadex G-25, coarse (8), and was also kept for incubations with [1-^{14}C]18:2(n-6) or [1-^{14}C]20:3(n-6). The microsomal fractions were resuspended using a 7-ml Dounce homogenizer in 4 ml of a cold solution containing 0.25 M sucrose and 0.15 M KCl, and adjusted to pH 7.2. The protein concentrations were measured by a modified Lowry method (9), with bovine serum albumin used as a standard. DNA concentrations were measured by the method of Halprin et al. (10) using calf thymus DNA (Type 1, sodium salt) from Sigma as standard.

Incubation procedure for desaturation

[1-^{14}C]18:2(n-6) and [1-^{14}C]20:3(n-6) were both diluted to a specific activity of 12.0 mCi/mmol with the corresponding unlabeled pure fatty acids. The desaturation experiments of the various epidermal microsomal preparations were performed in parallel with rat liver microsomal preparations as described previously (6), where

enzyme activities are well documented (7). Each of the ^{14}C-labeled fatty acids (100 nmol) was dissolved in ethanol and incubated, respectively, with the appropriate cofactors and either epidermal high speed supernatant (5.0 mg) or increasing concentrations of resuspended epidermal microsomal pellet (2.5–7.5 mg) corresponding to maximal rat liver microsomal Δ^6 and Δ^5 desaturase activities, as previously determined (6). The concentration of ethanol in the incubation mixture did not exceed 0.1%. The incubation mixture was preincubated for 5 min at 37°C. The reaction was initiated by the addition of the substrate. After aerobic incubation at 37°C for 25 min in a Dubnoff shaking incubator, the reactions were quenched by the addition of 10 ml of chloroform–methanol 2:1 (v/v). Control experiments were incubated with enzyme preparations previously boiled for 3 min.

Incubation procedure for chain elongation

[2-^{14}C]Malonyl CoA was diluted to a specific activity of 1.49 mCi/mmol with unlabeled malonyl CoA. The ^{14}C-labeled malonyl CoA was incubated with 100 nmol of unlabeled γ-linolenic acid (18:3(n–6)) with increasing amounts of protein (5.0–7.5 mg) from resuspended epidermal microsomal pellets. The elongation experiments with the various epidermal microsomal fractions were performed in parallel with rat liver microsomal preparations as previously described (11). The total incubation mixture of 2.0 ml contained KF (83.3 μmol), $MgCl_2$ (10 μmol), CoA (0.13 μmol), GSH (3.0 μmol), ATP (6.67 μmol), NADPH (1.33 μmol), malonyl CoA (0.13 μmol), and potassium phosphate buffer (80 μmol) at pH 7.2. The mixture was incubated anaerobically (in the presence of N_2) at 37°C for 25 min in a Dubnoff metabolic shaker. The reactions were quenched by the addition of 10 ml of chloroform–methanol 2:1 (v/v).

Identification of radioactive metabolites

After extraction of the incubation mixture with chloroform–methanol 2:1 (v/v) (12), the extracted lipids were transesterified with 6% methanolic–HCl at 75°C for 14–16 hr. To gain information on the nature of the unsaturation of the metabolites after incubations, portions of the radioactive products from elongase incubations were hydrogenated using PtO_2 as catalyst (13). The ^{14}C-labeled fatty acid methyl esters generated before and after reduction by PtO_2 were extracted with petroleum ether (boiling point 35–60°C) and separated by argentation thin-layer chromatography (TLC) and reverse phase HPLC. Argentation TLC on silica gel G impregnated with 10% $AgNO_3$ was performed as described previously (6), using the solvent system of diethyl ether–petroleum ether 100:70 (v/v) with methyl esters of 18:2(n–6), 18:3(n–6), 20:3(n–6), and 20:4(n–6) as references. Bands were detected under ultraviolet light after spraying with 0.2% 2',7'-dichlorofluorescein in ethanol. The ^{14}C-labeled methyl esters were quantitated using a TLC-proportional radioactivity scanner (Berthold, Model LB 2832, West Germany) equipped with an Apple IIe computer (Cupertino, CA).

Reverse phase high performance liquid chromatography

The ^{14}C-labeled fatty acid methyl esters were dissolved in acetonitrile, and separated on a reverse phase C18 ultrasphere ODS column, 5-μm particle size, 4.6 mm (I.D.) × 25 cm (Beckman, Berkeley, CA) using an isocratic solvent mixture of acetonitrile–water 90:10 (v/v), run for 30 min at a flow rate of 1.5 ml/min. Chromatography was done with a 324M system and a Model 421 microprocessor from Beckman Instruments. The ultraviolet absorption of the standards contained in the effluents was monitored continuously at 205 nm with a Beckman variable ultraviolet detector (model 165) which was coupled to an Altex C-R1A integrator–recorder. The ^{14}C was quantitated with a Flo-one™ (model HS) radioactivity flow detector using Flow-Scint II scintillation cocktail (Radiomatic Instruments, Tampa, FL). Counting efficiency was determined using [^{14}C]toluene standards (New England Nuclear).

Analysis of fatty acids in skin of control and psoriatic subjects

The skin specimens, removed as described previously, were minced and homogenized in a mixture of chloroform–methanol 2:1 (v/v) (12) with a Polytron (Model PT 10) homogenizer. The total lipids were transesterified and the methyl esters were analyzed using a Hewlett Packard gas–liquid chromatograph as previously described (6). Hydrogen gas flow rate was 1.75 ml/min, split ratio 1:70, flame ionization detector 250°C, and injector temperature 250°C. The oven temperature was operated isothermally at 200°C. Identification of methyl esters was established by comparison of retention data with those of known standards (Nu-Chek-Prep).

Statistical methods

Data were subjected to one-way analysis of variance using multiple comparisons (14), with the upper level of significance chosen at $P < 0.05$.

RESULTS

Δ^6 Desaturase activity by rat liver, normal, uninvolved and involved psoriatic skin enzyme preparations

Results of the incubations of [1-^{14}C]18:2(n–6) with the microsomal fractions obtained from rat liver and human epidermal preparations are shown in **Fig. 1 (A and B).** Incubations with rat liver preparations resulted in the formation of a radioactive product with a chromatographic mobility similar to reference 18:3(n–6) (Fig. 1A). Further

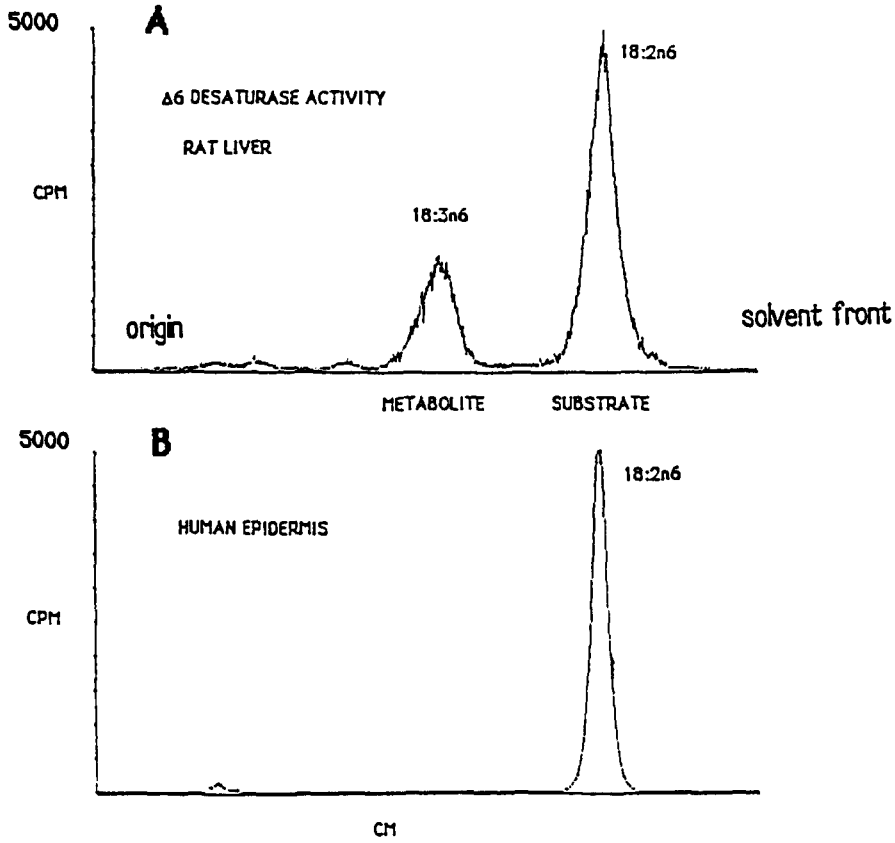

Fig. 1. Separation of radiolabeled fatty acid methyl esters by argentation thin-layer chromatography: Δ^6 desaturation. Conversion of [^{14}C]18:2(n-6) to [^{14}C]18:3(n-6) by microsomal preparations from rat liver and normal skin. [^{14}C]18:2(n-6) (100 nmol) was incubated with various concentrations of resuspended microsomal pellet in a 2.0-ml incubation mixture that contained ATP (6.67 μmol), CoA (0.13 μmol), NADH (1.2 μmol), $MgCl_2$ (10 μmol), GSH (3.0 μmol), KF (83.3 μmol), and potassium phosphate buffer (80 μmol) at pH 7.2. The mixture was incubated aerobically at 37°C for 25 min. Conversion of [^{14}C]18:2(n-6) to [^{14}C]18:3(n-6) was determined after transesterification of the total lipids as described in the text; cpm on the ordinate represents counts per minute; cm on the abscissa represents centimeters.

identification of radioactive 18:3(n-6) was achieved after reverse phase HPLC of the fatty acid methyl esters in the solvent system acetonitrile-water 90:10 (v/v) (chromatogram not shown). This observation is consistent with the findings of Marcel, Christiansen, and Holman (7), who demonstrated that the conversion of 18:2(n-6) into 20:4(n-6) proceeds according to the preferred sequence 18:2(n-6)→18:3(n-6). In similar incubations with increasing microsomal preparations from N, PU, and PI epidermis, no measurable conversion (<0.1%) of [1-^{14}C]18:2(n-6) to [^{14}C]18:3(n-6) (Δ^6 desaturase activity) could be demonstrated (Fig. 1B).

Δ^5 Desaturase activity by rat liver, normal, uninvolved, and involved psoriatic skin enzyme preparations

The lack of conversion of [1-^{14}C]18:2(n-6) to [^{14}C]18:3(n-6) by epidermal preparations prompted us to further investigate whether or not the Δ^5 desaturase activity was present in N, PU, or PI epidermal preparations. Incubations of microsomal fractions from rat liver used as a control with [1-^{14}C]20:3(n-6) resulted in the formation of a radioactive product with chromatographic properties similar to those of the reference 20:4(n-6) (**Fig. 2A**). The identity of this radioactive product was also corroborated using reverse phase HPLC. In similar incubations with increasing microsomal protein concentrations from N, PU, and PI epidermis, negligible conversion (<0.1%) of [1-^{14}C]20:3(n-6) into [^{14}C]20:4(n-6) was detected (Fig. 2B). These data demonstrate that N, PU, and PI epidermis lack Δ^5 desaturase activity.

Formation of [^{14}C]20:3(n-6) from [2-^{14}C]malonyl CoA and 18:3(n-6) by rat liver and normal, uninvolved, and involved psoriatic skin enzyme preparations

In preliminary experiments, the conversion of 18:3(n-6) into [^{14}C]20:3(n-6) by increasing concentrations of rat liver microsomal preparations was demonstrated as shown in **Fig. 3.** The results show that the formation of [^{14}C]20:3(n-6) was proportional to added microsomal protein up to 10 mg. It is evident that appreciable elongase

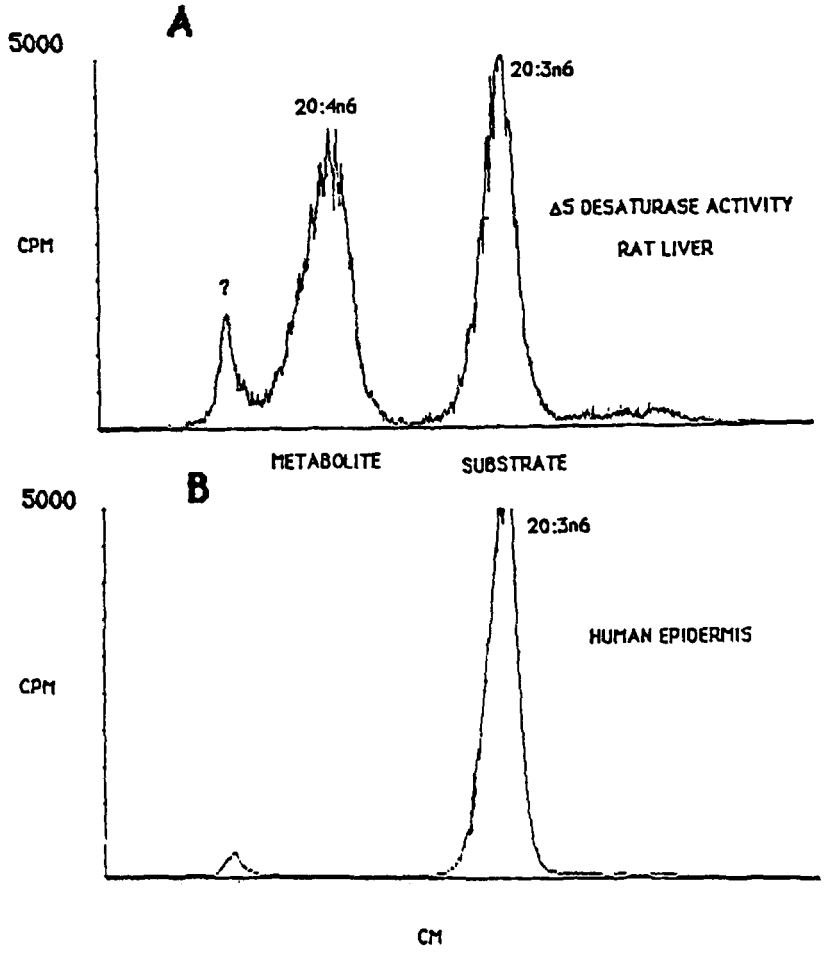

Fig. 2. Separation of radiolabeled fatty acid methyl esters by argentation thin-layer chromatography: Δ^5 desaturation. Conversion of [^{14}C]20:3(n-6) to [^{14}C]20:4(n-6) by microsomal preparations from rat liver and normal human skin. [^{14}C]20:3(n-6) (100 nmol) was incubated with various concentrations of resuspended microsomal pellet in a 2.0-ml incubation mixture that contained ATP (6.67 μmol), CoA (0.13 μmol), NADH (1.2 μmol), $MgCl_2$ (10 μmol), GSH (3.0 μmol), KF (83.3 μmol), and potassium phosphate buffer (80 μmol) at pH 7.2. The mixture was incubated aerobically at 37°C for 25 min. Conversion of [^{14}C]20:3(n-6) to [^{14}C]20:4(n-6) was determined after transesterification of the total lipids as described in the text.

activity was associated with microsomal protein levels of 5.0 to 10.0 mg. Because of the limited number of human skin biopsies, the epidermal elongase incubations were carried out using increasing concentrations (5.0–7.5 mg) of resuspended epidermal microsomal pellet. Radioactive products were characterized using argentation TLC and reverse phase HPLC.

A TLC chromatogram obtained from incubations of rat liver and human epidermal microsomal preparations with 18:3(n-6) and [2-^{14}C]malonyl CoA is shown in **Fig. 4 (A and B).** ^{14}C from [2-^{14}C]malonyl CoA was incorporated into radioactive products with chromatographic mobilities similar to those of 20:3(n-6) and palmitate (16:0). The metabolites were further characterized after reverse phase HPLC of the methylated metabolites (**Fig. 5A and B**). The data on the conversion of 18:3(n-6) into

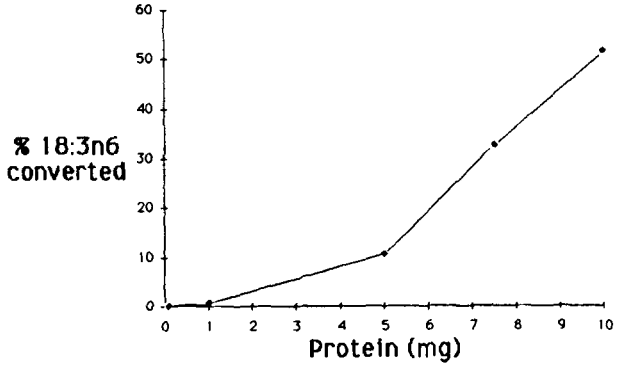

Fig. 3. The effect of increasing microsomal protein concentration on rat liver elongase activity. The results are expressed as percent of 18:3(n-6) converted to [^{14}C]20:3(n-6). Results are means from four separate experiments. Details of incubations and identification of radioactive products are described in the text.

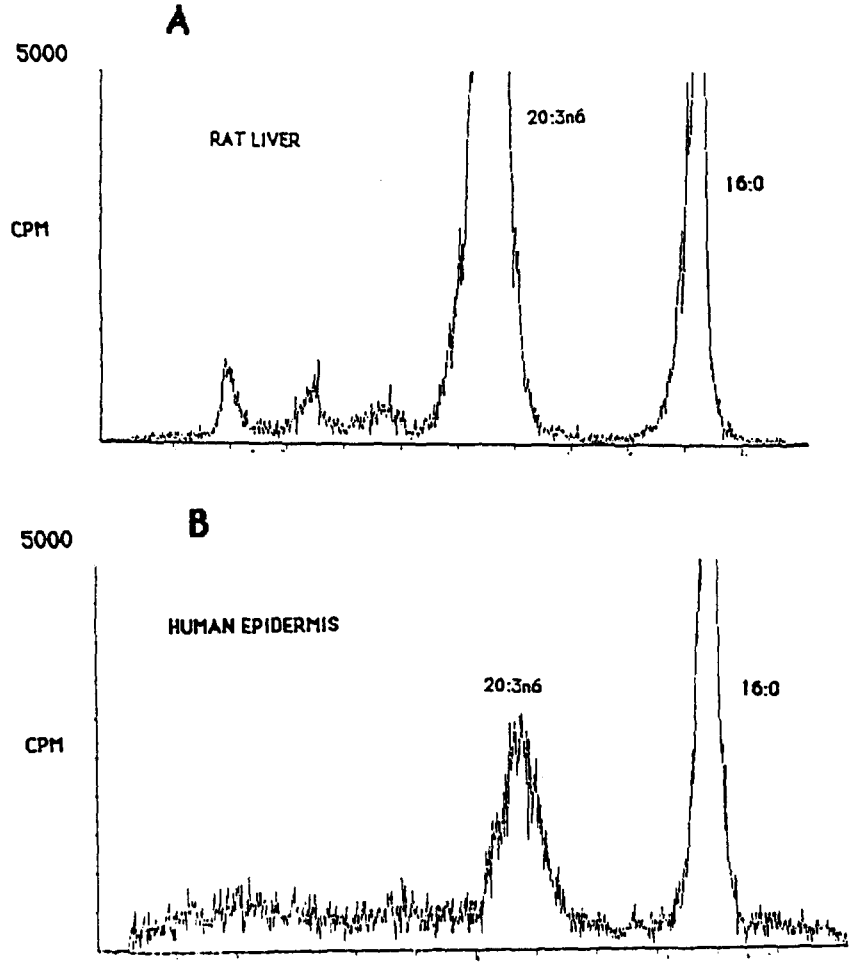

Fig. 4. Separation of radiolabeled fatty acid methyl esters by argentation thin-layer chromatography: elongase activity. Incorporation of [^{14}C]malonyl CoA into radioactive products using microsomal preparations from rat liver and normal human skin. [^{14}C]Malonyl CoA (0.2 μCi) and 18:3(n-6) (100 nmol) were incubated with various concentrations of resuspended microsomal pellet in a 2.0-ml incubation mixture that contained ATP (6.67 μmol), CoA (0.13 μmol), NADPH (1.33 μmol), $MgCl_2$ (10 μmol), GSH (3.0 μmol), KF (83.3 μmol), malonyl CoA (0.13 μmol), and potassium phosphate buffer (80 μmol) at pH 7.2. The mixture was incubated anaerobically at 37°C for 25 min. Conversion of 18:3(n-6) to [^{14}C]20:3(n-6) was determined after transesterification of the total lipids and extraction of the organic soluble components as described in the text.

[^{14}C]20:3(n-6) by the various epidermal preparations are summarized in **Table 1.** It is apparent than N, PU, and PI epidermal enzyme preparations are all capable of elongating 18:3(n-6). Of particular interest was the PI epidermal elongase activity which was significantly elevated above the N epidermal preparation ($P < 0.01$) and the PU epidermal preparation ($P < 0.05$) when normalized either by microsomal protein or DNA content.

Fatty acid composition of normal, uninvolved, and involved psoriatic epidermis

The fatty acid compositions of N, PU, and PI epidermal skin lipids are listed in **Table 2.** It is apparent that the weight percentage fatty acid composition of N versus PU epidermis is generally similar. Levels of 16:0 and stearate (18:0) are significantly depressed ($P < 0.01$ and $P < 0.05$, respectively) in the PI epidermis relative to N epidermis. The corresponding monoene products, palmitoleate (16:1(n-7)) and oleate (18:1(n-9)) were elevated, but only reaching a level of significance ($P < 0.05$) with 16:1(n-7). Interestingly, the level of lignocerate (24:0) in the PI epidermal strips was significantly depressed ($P < 0.01$) when compared to N epidermis, while the level of nervonate (24:1(n-9)) was strikingly elevated. The weight percent of the essential fatty acid 18:2(n-6) was depressed ($P < 0.01$) in the involved psoriatic group. Examination of 18:2(n-6) conversion products revealed that N, PU, and PI epidermis contained only negligible levels (<0.1 mg/100 mg of total fatty acids present) of 18:3(n-6). The levels of epidermal 20:3(n-6) were similar, while the levels

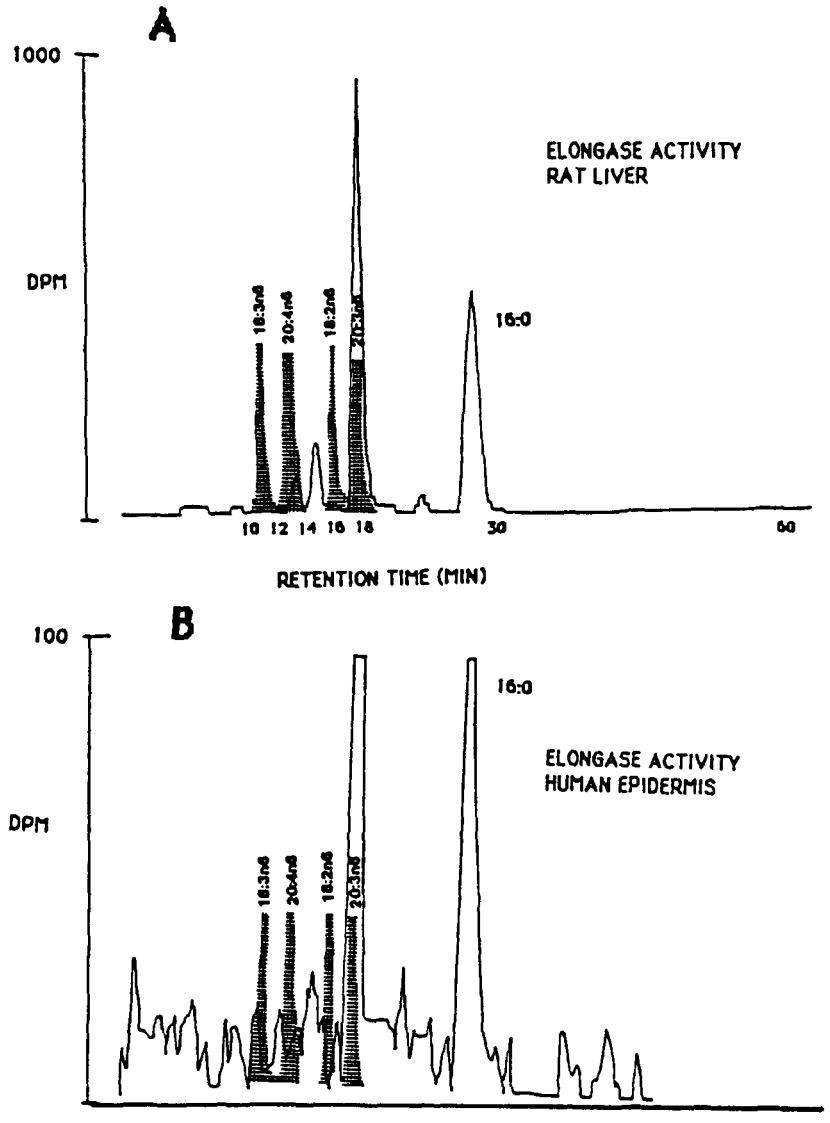

Fig. 5. Separation of radiolabeled fatty acid methyl esters by reverse phase high-performance liquid chromatography: elongase activity. Incorporation of [^{14}C]malonyl CoA into radioactive products using microsomal preparations from rat liver and normal human skin. Details of incubations are described in Fig. 4. The solid continuous line represents a profile of the radioactive metabolites. The shaded areas represent the positions of the unlabeled carrier standards.

of 20:4(n-6) were lower in the PI epidermis relative to normal, although the differences were of borderline significance (P = 0.076).

DISCUSSION

Data from these studies have demonstrated the inability of PI, PU, and the N epidermal preparations to desaturate both 18:2(n-6) and 20:3(n-6) acids, indicating the lack of Δ^6 and Δ^5 desaturases in the human epidermis. It is unlikely that the absence of epidermal desaturase activity is the result of freezing of the tissue specimen or contamination of the microsomal preparations because, in a previous study, we demonstrated that microsomal preparations from fresh as well as frozen rat and guinea pig epidermia (6) lacked the capacity to transform 18:2(n-6) into 20:4(n-6). Furthermore, in separate experiments, we noted that epidermal fractions containing both lysosomes and mitochondria did not significantly influence rat liver desaturase activity (unpublished results). Therefore, these data are consistent with a recent report of the lack of Δ^6 and Δ^5 desaturase activites by microsomal preparations from rat and guinea pig epidermis (6). They also indicate that the 20:4(n-6) found principally esterified to membrane phospholipids in the epidermis is not biosynthesized locally from tissue 18:2(n-6) and must, therefore, be derived from another endogenous source. Although

TABLE 1. Elongation of 18:3(n-6) by epidermal enzyme preparations

Tissue Source	pmol 20:3(n-6) Formed per mg protein per min	pmol 20:3(n-6) Formed per mg DNA per min
Normal epidermis (n = 4)	2.87 ± 0.12[a]	44.05 ± 2.96[b]
Uninvolved epidermis (n = 2)	3.10 ± 0.57[c]	47.00 ± 11.74[d]
Involved epidermis (n = 2)	19.02 ± 4.19[a,c]	335.40 ± 73.90[b,d]

The results are expressed as either pmol 20:3(n-6) formed per mg protein per min or pmol 20:3(n-6) formed per mg DNA per min after correction for boiled enzyme control. Details of incubation and separation of radiometabolites are described in the text. The data are means ± SD from two to four incubations.

Values with similar superscripts denote a statistical difference at leves of $P < 0.01$[a,b] and $P < 0.05$[c,d], respectively, using a one-way analysis of variance with multiple comparisons.

the mode of transport of 20:4(n-6) to the epidermis is unknown, it has been assumed that nondietary EFAs in the skin originate from the liver and are transported to the skin by the plasma lipoproteins (15). Clarification of the 20:4(n-6) transport process to the skin must await further studies.

The epidermal desaturase data are interesting in view of the fact that 20:4(n-6) metabolites participate in the inflammatory and proliferative processes in psoriasis (2, 3). Our studies indicate that PU and PI epidermis are not capable of locally biosynthesizing 20:4(n-6) from tissue 18:2(n-6), in view of the lack of both Δ^6 and Δ^5 desaturase activities. Forster et al. (16) have reported that the increased 20:4(n-6) metabolites in PU and PI skin (3, 4) may be due to an enhanced epidermal phospholipase A_2 activity, in the absence of a compensating level of acyl transferase, thus leading to an elevated concentration of free 20:4(n-6), which in turn could result in the reported increased concentrations of eicosanoids (17). Consistent with these observations are the results from the analysis of the fatty acid composition of total lipids in the epidermis (Table 2). Although the levels of free 20:4(n-6) were not measured, the relative levels of total (free and esterified) 20:4(n-6) were depressed in the PI epidermis when compared to either N or PU epidermis. It is therefore likely that the increased concentration of eicosanoid in both PU and PI epidermis is due to an increased concentration of free 20:4(n-6) released from tissue phospholipids and not total 20:4(n-6) produced through the local conversion of epidermal 18:2(n-6) to 20:4(n-6).

Incubation of [2-^{14}C]malonyl CoA and 18:3(n-6) with microsomal preparations from skin specimens from N, PU, and PI epidermis revealed the presence of elongase activity capable of converting 18:3(n-6) to [^{14}C]20:3(n-6) (Table 1). Examination of the epidermal fatty acid composition revealed negligible amounts of 18:3(n-6) but significant levels of 20:3(n-6), suggesting the presence of local elongase activity. Interestingly, the elongase activity was significantly elevated in the PI epidermis. The reason for the strikingly elevated activity in PI preparations is

TABLE 2. Fatty acid composition of total lipids in human epidermis

Fatty Acid Methyl Esters	Normal	Psoriatic	
		Uninvolved	Involved
14:0[a]	1.11 ± 0.55	1.45 ± 0.99	0.84 ± 0.56
16:0	14.01 ± 1.48[b]	13.93 ± 1.00[c]	11.97 ± 1.52[b,c]
16:1(n-7)	2.34 ± 1.05[d]	2.58 ± 1.14	3.85 ± 2.17[d]
18:0	11.06 ± 0.86[d]	10.86 ± 0.64	10.14 ± 1.28[d]
18:1(n-9)	15.12 ± 1.88	13.74 ± 1.26[b]	16.80 ± 3.15[b]
18:2(n-6)	21.52 ± 2.79[b]	21.03 ± 1.98[c]	15.65 ± 2.85[b,c]
18:3(n-6)	—[e]	—	—
20:0	1.58 ± 0.21	1.72 ± 0.46	1.75 ± 0.56
20:3(n-6)	1.46 ± 0.22	1.41 ± 0.50	1.42 ± 0.55
20:4(n-6)	6.22 ± 1.82	6.46 ± 0.97[d]	5.00 ± 1.41[d]
22:0	2.68 ± 0.45[c]	2.92 ± 0.29[b]	1.40 ± 0.66[b,c]
24:0	10.00 ± 1.85[c]	10.47 ± 1.83[b]	4.17 ± 0.58[b,c]
24:1(n-9)	—[b]	0.43 ± 1.16[b,c]	4.27 ± 2.25[b,c]

The results are expressed as mg/100 mg of total fatty acids present. Each figure represents the mean ± SD for normal (n = 12), uninvolved psoriatic (n = 13), and involved psoriatic (n = 12). Details of extraction and separation are described in the text.

[a]Only the major fatty acids are listed. The number after "n" indicates the number of carbon atoms from the methyl end of the acyl chain to the nearest double bond.

[b,c,d]Values with similar superscripts denote a statistical difference at the level of $P < 0.01$[b,c] and $P < 0.05$[d], respectively, using a one-way analysis of variance with multiple comparisons.

[e]Less than 0.10 mg/100 mg of total fatty acids.

presently unclear. The difference manifested by the PI preparations is unlikely to be due to the occasional contamination of the PI preparation by infiltrating leukocytes, since the chain elongation process in polymorphonuclear leukocytes is very limited (18, 19). Our data, therefore, support the concept that the elongase-like activity, as demonstrated by the transformation of 18:3(n-6) into [^{14}C]20:3(n-6), is very likely enhanced in the PI epidermis. Although the function of the elongase in the human integumentary system remains to be elucidated, a notable feature of 20:3(n-6) is its possible conversion to the l-series of prostaglandins (20). These metabolites are largely thought to possess anti-phlogistic properties (21, 22). Kassis and Sondergaard (23) have utilized radioimmunoassay to determine PGE_1 levels in normal human skin but no data as yet are available on PGE_1 activity in PU and PI epidermis.

The present study also confirms that by far the most abundant EFAs in human skin are 18:2(n-6) and 20:4(n-6) (15). However, the decrease in the levels of 18:2(n-6) in PI epidermis (Table 2) in our studies is unclear in view of the absence of a Δ^6 desaturase activity. A similar depression of 18:2(n-6) has been reported in PI plasma lipid esters (24). The possibility that 18:2(n-6) is metabolized to other oxygenated products remains very likely. This view is consistent with a report by Camp et al. (25), who demonstrated that extracts of scale and chamber fluid from abraded psoriatic patients contained 13-hydroxy-octadecadienoic acid (13-HODD) and 9-hydroxy-octadecadienoic acid (9-HODD), both mono-oxygenated products of 18:2(n-6). Furthermore, in a recent study of the metabolism of 18:2(n-6) by rat epidermis, Nugteren et al. (26) suggested the possibility that 18:2(n-6) is carried into the epidermal barrier layer by polar lipids where it is converted into peroxidated lipids by lipoxygenase reactions. The elevated levels of 16:1(n-7) and 18:1(n-9) in combination with a depression of their saturated antecedents, 16:0 and 18:0, respectively, as revealed in Table 2 suggest a possible induction of the Δ^9 desaturase. This observation is interesting in view of the report of Cooper, McGrath, and Shuster (27), who demonstrated that the rate and pattern of epidermal lipogenesis from [^{14}C]glucose was raised in psoriatic lesions when compared with matched uninvolved epidermis. Further studies are necessary to determine whether 18:1(n-9) can substitute for 18:2(n-6) in acylglucosyl ceramide in psoriatic epidermis. The significance of the depressed level of 18:2(n-6) in lesional epidermis remains to be determined.

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