Conversion of linoleic acid into arachidonic acid by cultured murine and human keratinocytes

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Abstract The origin of arachidonic acid (AA) found in the epidermis is not known. Two possibilities exist: either de novo synthesis within the epidermal keratinocyte, or transport of AA formed at distant tissue sites. The current study examined the ability of cultured murine and human keratinocytes to metabolize exogenously added linoleic acid (LA). Conversion of radiolabeled substrate (^{14}C-LA) into 18:3(n-6), 20:2(n-6), 20:3(n-6), and 20:4(n-6) (AA) was noted. The conversion of non-radiolabeled 18:3(n-6) or 20:2(n-6) was also examined and the pattern of metabolites synthesized suggests that the preferred metabolic pathway for conversion of linoleic acid into arachidonic acid is via the classically described pathway in which a Δ^6 desaturase constitutes the initial reaction. Although cultured skin fibroblasts are known to convert linoleic acid into arachidonic acid, the current study demonstrates that cultured epidermal keratinocytes can also avidly metabolize exogenous linoleic acid. The ability of cultured keratinocytes, and not of whole epidermis in vivo, to convert linoleic acid into arachidonic acid suggests that specific enzymatic activities may be induced by the tissue culture system itself. Hence, findings of metabolic capabilities in cultured cells may not necessarily be extrapolated to the in vivo situation.—**Isseroff, R. R., V. A. Ziboh, R. S. Chapkin, and D. T. Martinez.** Conversion of linoleic acid into arachidonic acid by cultured murine and human keratinocytes. *J. Lipid Res.* 1987. **28:** 1342–1349.

Supplementary key words desaturation • elongation • epidermis • skin • polyunsaturated fatty acid

The observations by Burr and Burr in 1930 (1), that multisystem disease occurs in animals deprived of polyunsaturated fatty acids of the n-6 series, initiated the concept of the essentiality of these fatty acids (FA). When, however, the essential FA precursor,18:2(n-6), is exogenously supplied, it can be further metabolized in many tissues by a series of desaturation and elongation steps to 20:4(n-6) (2), which itself serves as a precursor for the genesis of many important eicosanoid (prostaglandin and leukotriene) mediators. Athough these mediators are found within the skin (3) and, indeed, are believed to play regulatory roles in the normal differentiation process (4) as well as the pathogenesis of specific diseases (5), the site of origin of the parent 20:4(n-6) FA present in the skin is unknown. Recent reports by Chapkin and Ziboh (6) and Chapkin et al. (7) indicate that human, guinea pig, and rat epidermis are unable to transform exogenously supplied 18:2(n-6) into 20:4(n-6), and suggest that the 20:4(n-6) found in this tissue is transported from the other sites.

Studies using tissues excised from whole animals, however, are subject to complex hormonal interactions, and the products generated may represent metabolic contributions of the many cell types present within the tissue. For these reasons, some investigators have chosen to study the pathways of conversion of 18:2(n-6) utilizing in vitro tissue culture systems. Studies of cells cultured in defined media under controlled conditions have determined that many cell types can actively convert 18:2(n-6) by the classical desaturation/elongation pathway: 18:2(n-6) (9, 12) → 18:3(n-6) (6, 9, 12) → 20:3(n-6) (8, 11, 14) → 20:4(n-6) (5, 8, 11, 14) (8, 9). On the other hand, a number of cell types have been found to be lacking some of the desaturase activities and are, therefore, unable to convert 18:2(n-6) into 20:4(n-6) (10, 11).

The current study investigates the fate of exogenously supplied 18:2(n-6) in cultured epidermal keratinocytes. The biosynthesis of 20:4(n-6) in these cultured cells is observed, and the pattern of the intermediate metabolites synthesized suggests that these cultured cells utilize the classically described pathway in which the Δ^6 desaturase constitutes the first conversion step (2). The ability of cultured keratinocytes and not, as reported previously, whole skin to accomplish this conversion (6, 7) suggests that the cell culture system itself may induce metabolic alterations within the cells, and that extrapolation of data generated by cell culture systems into the in vivo situation should be undertaken with caution.

MATERIALS AND METHODS

Cell culture techniques

Neonatal mouse keratinocyte cultures were established following the technique of Yuspa and Harris (12). Mouse skins were removed, epidermis was separated from dermis by trypsin flotation, and a cell suspension was prepared from the resultant epidermal sheets. Viable keratinocytes were plated at a density of 2.5×10^5 cells/cm^2 in Dulbecco's modified Eagle's medium (DMEM) (Flow Laboratories, McLean, VA) supplemented with 10% fetal calf serum (FCS) (Irvine Scientific, Santa Ana, CA) and maintained at 37°C in an atmosphere of 95% air and 5% CO$_2$. After achieving confluence (generally 24 hr post-plating) cultures were washed and incubated in serum-free DMEM containing 0.025% fatty acid-free (FAF) bovine serum albumin (Boehringer-Mannheim, Indianapolis, IN) and 0.25 μCi of [1-^{14}C]linoleic acid (58 mCi/mmol) (Amersham, Arlington Heights, IL) for 16 hr at 37°C. After washing off unincorporated radiolabel three times with phosphate-buffered saline (PBS), some plates were immediately processed for lipid extraction; others were washed as outlined above to remove unincorporated radiolabel and further incubated in DMEM + 10% FCS at 37°C for a total of 64 hr. Control dishes containing medium and radiolabel but no cells were also incubated and processed for lipid extraction. Replicate culture plates were treated with trypsin and total cell number was enumerated. In studies utilizing non-radiolabeled precursors, ethanolic solutions of the fatty acids, 18:3(n-6) or 20:2(n-6), were added to the culture media (DMEM + 10% FCS) at a final concentration of 75 μM when the cultures were initially plated. Fatty acids (18:3(n-6) and 20:2(n-6)) were obtained from Nu-Chek-Prep, Elysian, MN. Control cultures received unsupplemented culture medium (DMEM + 10% FCS). Cultures were processed for lipid extraction at 18 and 64 hr post-addition of the fatty acid.

Murine fibroblast cultures were initiated from explants of scissors-minced sections of the trypsin-separated dermal component of the mouse skin and incubated in DMEM + 10% FCS. When fibroblast outgrowth was evident, culture plates were treated with trypsin to obtain a single cell suspension which was subsequently replated and further incubated in DMEM + 10% FCS. When culture dishes attained confluence, they were radiolabeled and processed as outlined above for keratinocyte cultures.

Human keratinocyte cultures were initiated from neonatal foreskins, utilizing the method of Rheinwald and Green (13). Briefly, a single-cell suspension of keratinocytes obtained from trypsin-separated epidermis was plated in culture dishes preseeded with a feeder layer of mitomycin-C-treated 3T3 cells (clone CCL92, American Type Culture Collection, Rockville, MD) in DMEM containing 10% FCS, 0.4 μg/ml hydrocortisone, 10 ng/ml cholera toxin, and 1 ng/ml epidermal growth hormone. At 50% confluence, the primary cultures were sprayed with 0.02% EDTA to remove any contaminating fibroblasts, and new mitomycin-C-treated 3T3 cells were added. Cultures were subcultured at confluence onto dishes that had been preseeded with feeder 3T3 cells. The repeated subcultivation and weekly treatment of these cultures with 0.02% EDTA freed the plates of any contaminating fibroblasts, yielding pure keratinocyte cultures. After attaining confluence (generally 3–4 weeks of culture) cells were radiolabeled and processed at various times after labeling as outlined above.

Extraction of lipids

Cultured cells were scraped from the culture dish in 1 ml of chilled methanol and extracted by the method of Folch, Lees, and Sloane Stanley (14). Tissue samples and control media were similarly extracted in 19 volumes of chloroform–methanol 2:1 (v/v). Aliquots of cellular extracts were removed for protein analysis, and the organic phases of the extracts were filtered, dried under N$_2$, and stored at -20°C untile further analysis.

Analysis of lipid extracts

Thin-layer chromatographic (TLC) analyses. Fatty acid methyl esters (FAMEs) prepared from total lipid extracts utilizing methanolic HCl (15) were applied along with cold carrier FAMEs of 18:2(n-6), 18:3(n-6), 20:3(n-6), and 20:4(n-6) to silica gel G (Merck, FRG) plates impregnated with AgNO$_3$ and developed as outlined by Barrett, Dallas, and Padley (16). The gel was scraped from the plate at 5-mm intervals into vials containing 12 ml of Econofluor (New England Nuclear, Boston, MA) and radioactivity in each fraction was quantitated by liquid scintillation spectrometry.

Gas–liquid chromatographic (GLC) analysis. FAMEs prepared as outlined above, were analyzed on a Hewlett-Packard 5730A gas chromatograph equipped with a flame ionization detector system, using a fused silica capillary column (DB-225, J & W. Scientific, Rancho Cordova, CA). The column temperature was maintained at 200°C and the carrier flow rate was 1.76 ml/min. The FAMEs were identified by comparison of retention times to those of known standards (Nu-Chek-Prep, Elysian, MN) and relative percentages were calculated by integration of the area under each peak using the Hewlett-Packard 3380A Integrator. Values were compared using the paired, two-tailed Student's t test.

High pressure liquid chromatographic (HPLC) analysis. For HPLC analysis of FAMEs, the method of Aveldaño, Van Rollins, and Honocks (17) was utilized; 10^5 cpm of specific FAME obtained from preparative argentation TLC of FAMEs was dissolved in 25 μl of acetonitrile and injected into a reverse phase 25 cm $\times$ 4.6 mm column packed with 5 μm Ultrasphere ODS (Altex, Irvine, CA). The HPLC was carried out on a Beckman system equipped with a Model 421 controller, 100A pump, 165 variable wavelength detector set at 205 nm, and a Flo-One radioactivity flow

detector (Radiometic Instruments and Chemical Co., Tampa, FL). The mobile phase used was a solvent mixture of acetonitrile–water 9:1. The flow rate was 1.5 ml/min and counting of ^{14}C-labeled samples was done using a 1:3 ratio of eluant–cocktail (Flo-Scint II, Radiometric Instruments, Tampa, FL).

Fatty acids were also derivatized to form p-bromophenacyl esters, following the method of Borch (18) and resolved on HPLC reverse phase chromatography as outlined above with the wavelength scanner set at 254 nM. Percentage conversion of substrate was calculated using the formula: % conversion = (cpm product/cpm product + substrate) × 100. The calculations were corrected by subtraction of cpm present within nonspecific degradation products detectable in control medium that had been incubated at 37°C for 16 hr with ^{14}C-labeled substrate in the absence of cells.

Immunoperoxidase staining techniques

To investigate the possibility of fibroblast contamination of keratinocyte cultures, immunoperoxidase staining for vimentin, the cytoskeletal protein of fibroblasts, was carried out. Murine or human keratinocytes were plated, using the aforementioned techniques, onto glass coverslips and cultivated as outlined above until they reached a density of 70% confluence. After fixation with MeOH at 4°C, coverslips were stained utilizing standard immunoperoxidase technique (19) using a rabbit–polyclonal anti-vimentin antibody (20) (generously supplied by Dr. R. Hynes of Massachusetts Institute of Technology) as the primary antibody and a goat-anti-rabbit peroxidase-conjugate (Cappel Laboratories, Westchester, PA) as the secondary antibody. Positive controls consisted of the 3T3 line of mouse fibroblasts, which contain vimentin.

RESULTS

Previous analysis of phospholipid fatty acids in murine keratinocytes cultivated for 5 days in 10% FCS-containing medium had demonstrated a fourfold decrease in the level of linoleic acid (LA) as compared to noncultured cells (21). The current study reveals that this decrease is a gradual one; levels of LA drop gradually from 13% of total fatty acids in noncultured cells to 3.6% of the total by day 5 of culture (**Fig. 1**). This probably results from the relative paucity of linoleic acid in the fetal calf serum present in the tissue culture medium. The concentration of linoleic acid present in the total lipids of the fetal calf serum used for these studies was 0.07 mM. The serum was used at a 10% final concentration, which results in a final tissue culture concentration of linoleic acid which is 40–50 times below that normally found in mouse serum (21). Interestingly, the levels of arachidonic acid in the cultured cells did not fall with cultivation. Lipids of noncultured, as well as of cells cultured from 1–5 days, contained approximately 4–5% of
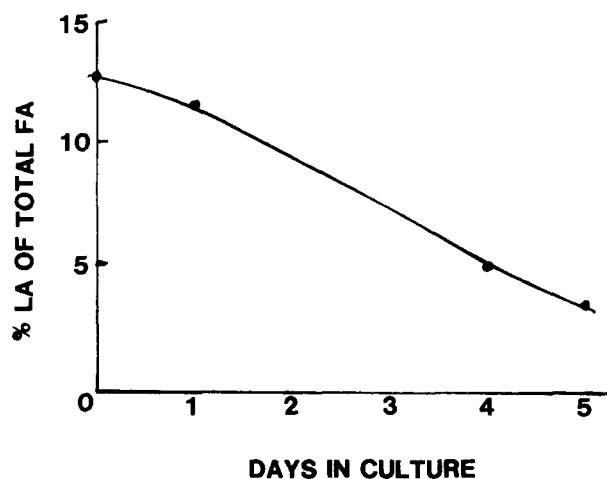

Fig. 1. Percentage of linoleic acid of total cellular fatty acids in keratinocytes maintained in 10% FCS-containing culture medium. Cellular lipids were extracted and constituent fatty acids were analyzed as outlined in Methods.

total fatty acids as arachidonic acid (AA) (21). Three possible explanations for this relatively stable level of AA can be postulated: either AA is incorporated from the FCS in the culture medium, de novo synthesis from precursor LA occurs, or there is decreased metabolism and utilization of AA.

To differentiate among these possibilities and to determine whether the cultured cells had the metabolic capability to convert LA into AA, keratinocyte cultures that had attained confluency in DMEM + 10% FCS were incubated for 18 hr with serum-free medium containing [^{14}C]linoleic acid at a final concentration of 1.6 μM. Preliminary analysis of the metabolically labeled fatty acids extracted from the cultures by TLC (**Fig. 2**) revealed time-dependent increases in the accumulation of radiolabel in products that
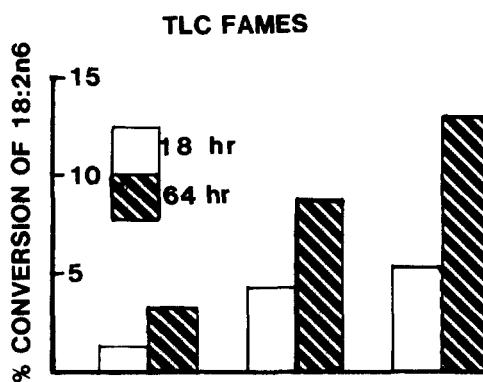

Fig. 2. Thin-layer chromatographic analysis of radiolabeled conversion products of [^{14}C]18:2(n-6) substrate by cultured murine keratinocytes. Confluent keratinocyte cultures were incubated with 0.25 μCi [^{14}C]18:2(n-6) for 18 hr. Extracted lipids were analyzed for incorporation of radiolabel at 18 hr (clear bars) and 64 hr (hatched bars) post-incubation by TLC of FAMES. Percent conversion = (cpm product/cpm product + substrate) × 100.

co-chromatographed with authentic standards of the intermediates 18:3(n-6), 20:3(n-6), and 20:4(n-6) fatty acids. By 64 hr after incubation, 12.9% of the incorporated ^{14}C label had accumulated in 20:4(n-6).

To confirm these results, two other chromatographic systems were employed to separate the radiolabeled products. HPLC analysis of the radiolabeled FAMEs (**Fig. 3A**) also demonstrated a time-dependent conversion of the 18:2(n-6) substrate into radioactive products which co-eluted with authentic standards of 20:3(n-6) and 20:4(n-6), as did HPLC analysis of p-bromophenacyl esters of the cellular fatty acids (Fig. 3B). Conversion of substrate into 20:4(n-6) accounted for 16.3%, by HPLC analysis, of FAMEs and 20%, by analysis of p-bromophenacyl derivatives, of total

radioactivity incorporated in cellular lipids at 64 hr. Hence, although there was slight variation in the calculated percentage conversion with the different methodologies, all three clearly demonstrated the time-dependent increased accumulation of radiolabel into the 20:4(n-6) product.

The ability of isolated, cultivated dermal fibroblasts to convert the 18:2(n-6) substrate into the 20:4(n-6) product was also examined. Cultures of these cells, initiated from the dermal components of mouse skin that had attained confluency in 10% FCS-containing medium, were incubated with radiolabeled LA in a fashion analogous to the keratinocyte cultures. **Fig. 4** reveals that these cells demonstrated a time-dependent conversion of substrate into the products chromatographically identical to 20:4(n-6) and intermediate products. Again, smaller accumulations of label are noted in the 18:3(n-6), a Δ^6 desaturase product. Approximately equivalent amounts of conversion products were detected by HPLC analysis of both the FAME and p-bromophenacyl ester derivatives (37% and 45% conversion of 18:2(n-6) into 20:4(n-6) by 64 hr, respectively).

To assure that the conversion of 18:2(n-6) into 20:3(n-6) and 20:4(n-6) observed in the mouse keratinocyte cultures was the result of the metabolic activity of the keratinocytes and not the contribution of possible contaminating fibroblasts, the numbers of fibroblasts present within the keratinocyte cultures were enumerated. Keratinocyte cultures were stained using immunoperoxidase techniques utilizing an antibody to vimentin, a cytoskeletal intermediate filament protein which is absent in keratinocytes and present in fibroblasts. Analysis of multiple cultures revealed that no greater than 1:1,000 cells stained positive for vimentin. Hence, the keratinocyte cultures utilized for these studies contained few contaminating fibroblasts, and it is unlikely that the total conversion of substrate observed in the keratinocyte cultures represents the metabolic product of fewer than 1:1000 cells.

To further investigate the possibility of contribution of metabolic products by contaminating fibroblasts, human keratinocyte cultures were examined. These cultures have a much longer life-span (4–6 weeks as compared to 1 week for mouse keratinocytes) and any faster growing contaminating fibroblasts can be more easily observed and eradicated. These cultures were initially co-cultivated with mitomycin C-treated 3T3 cells, which suppress the growth of contaminating fibroblasts. In addition, these cultures were subcultivated and re-treated weekly with 0.02% EDTA, which selectively removes any contaminating fibroblasts and/or remaining 3T3 cells (22). Analysis of these cultures by immunoperoxidase techniques revealed no contaminating vimentin-positive cells. Hence, this human keratinocyte system provided a purer keratinocyte population in which to evaluate conversion of LA substrate.

Fig. 5 demonstrates that when these cells were incubated with ^{14}C-labeled 18:2(n-6) substrate, both of the labeled products that co-chromatographed with 20:3(n-6) and
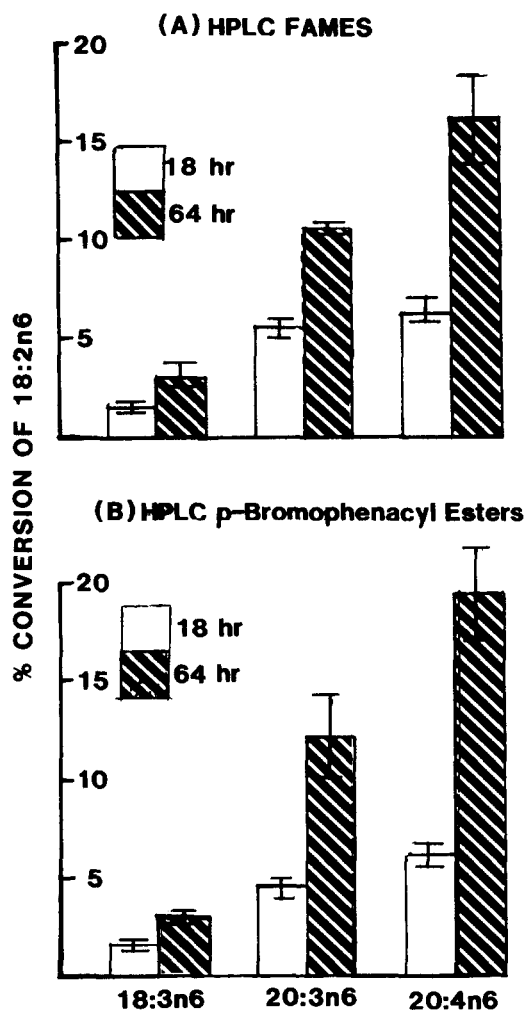

Fig. 3. HPLC analysis of radiolabeled conversion products of [^{14}C]18:2(n-6) substrate by cultured murine keratinocytes. Confluent keratinocyte cultures were incubated and lipids were extracted as for Fig. 2, at 18 hr (clear bars) and 64 hr (hatched bars) post-incubation. HPLC of FAMES (panel A) and HPLC of p-bromophenacyl ester derivatives (panel B). Percent conversion = (cpm product/cpm product + substrate) × 100. Values represent means of two separate experiments; bars represent range.
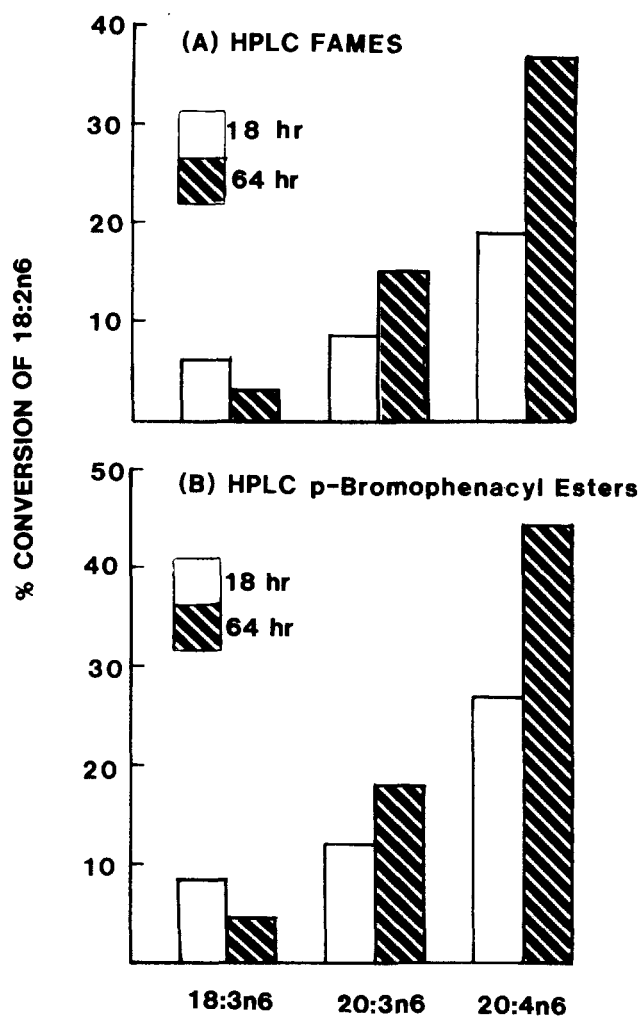
(A) HPLC FAMES

18 hr
64 hr

% CONVERSION OF 18:2n6

(B) HPLC p-Bromophenacyl Esters

18 hr
64 hr

18:3n6   20:3n6   20:4n6

Fig. 4. Chromatographic analysis of radiolabeled conversion products of [^{14}C]18:2(n-6) substrate by cultured murine dermal fibroblasts. Protocol and analysis of products as outlined in Fig. 2. HPLC of FAMES (panel A) and of p-bromophenacyl esters (panel B), clear bars, 18 hr incubation; hatched bar, 64 hr incubation.

zation of this intermediate into further metabolic products. A third possibility was that the 18:2(n-6) precursor was being metabolized by an alternate pathway in which the initial reaction was that of elongation to 20:2(n-6) rather than desaturation to 18:3(n-6). This "optional" pathway (**Fig. 6**) has been described in liver (12) as well as in other epithelial tissues such as human colon and bladder (23). To distinguish between these possibilities, murine keratinocyte cultures were supplemented with non-radiolabeled precursors, either 18:3(n-6) or 20:2(n-6) (final concentration of 75 μM) for 18 and 64 hr. Analysis of fatty acid profiles of the cultures (**Table 1**) indicates that only cultures supplemented with 18:3(n-6) demonstrated significant increases in the presence of intermediates 20:3(n-6) and 20:4(n-6) present in cellular lipids. In contrast, cultures supplemented with 20:2(n-6) demonstrated no increase in 20:3(n-6) and, in fact, a small decrease in 20:4(n-6) content. Of note is the marked increase in cellular 18:2(n-6) in cultures supplemented with 20:2(n-6). This latter finding suggests that rather than being metabolized to 20:4(n-6), added 20:2(n-6) may be retroconverted to 18:2(n-6) by the cells, a phenomenon suggested by Sprecher and Lee (24), and Stearns, Rysary, and Privett (25).

DISCUSSION

The site of synthesis of the parent 20:4(n-6) compound found in the skin is of considerable importance because of the known broad biological effects the derived eicosanoid metabolites have in this tissue (4, 5). To investigate the ability of the epidermal portion of the skin to generate 20:4(n-6) from exogenous 18:2(n-6) we utilized a cell culture system.
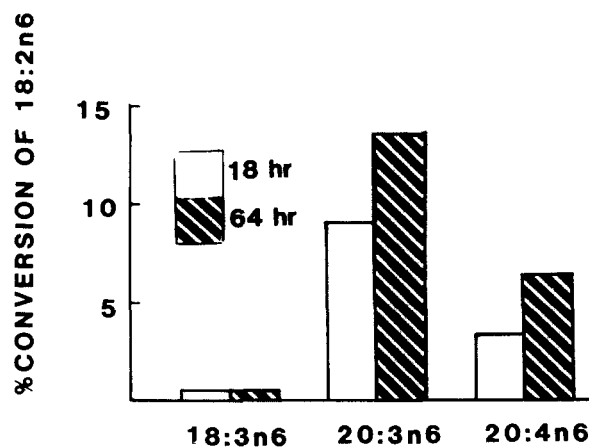
20:4(n-6) were formed in a time-dependent manner. There was no time-dependent increase in the accumulation of radiolabel into the 18:3(n-6) product; radioactivity that co-chromatographed with 18:3(n-6) standard accounted for approximately 1% or less of total radioactivity incorporated. Therefore, cultivated human keratinocytes were able to transform the precursor 18:2(n-6) into the 20:4(n-6) product as could the murine cells, demonstrating that the observed enzymatic activities were not species specific.

Of particular interest is the lack of increased accumulation, with time, of radiolabel into 18:3(n-6) in the human keratinocyte cultures (Fig. 5) and the relatively low rates of accumulation of this intermediate in the murine keratinocyte cultures (Figs. 2 and 3). This suggests that the radioactivity associated with this fraction may represent either a very low rate of synthesis or, alternatively, the rapid utili-

% CONVERSION OF 18:2n6

18 hr
64 hr

18:3n6   20:3n6   20:4n6

Fig. 5. Chromatographic analysis of radiolabeled conversion products of [^{14}C]18:2(n-6) substrate by cultured human keratinocytes. Confluent human keratinocyte cultures were incubated with radiolabeled substrate for 18 hr (clear bars) and 64 hr (hatched bars), at which time cellular lipids were extracted and transmethylated; radiolabeled FAMES were analyzed by HPLC, as outlined in Figure 2B.
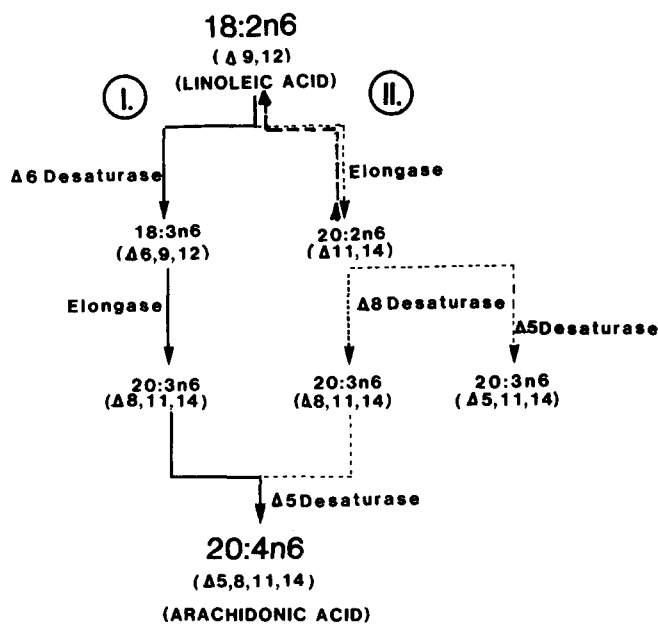
18:2n6
(Δ 9,12)
(LINOLEIC ACID)

I.   II.

Δ6 Desaturase   Elongase

18:3n6   20:2n6
(Δ6,9,12)   (Δ11,14)

Elongase   Δ8 Desaturase   Δ5 Desaturase

20:3n6   20:3n6   20:3n6
(Δ8,11,14) (Δ8,11,14) (Δ5,11,14)

Δ5 Desaturase

20:4n6
(Δ5,8,11,14)
(ARACHIDONIC ACID)

Fig. 6. Alternate pathways for the metabolism of 18:2(n-6) by cultivated keratinocytes. Pathway I: classical pathway, Δ^6 desaturase constitutes first step. Pathway II: "optional" pathway, elongase constitutes first step. Dashed lines (----) indicate "optional" pathway of enzymatic reactions, not active in these cells. Broken line (− −) represents suggested retroconversion of 20:2(n-6) into 18:2(n-6).

This system confers the advantages of a controlled environment, free of complex hormonal interactions found in vivo. Under these conditions, both human and murine epidermal keratinocyte cultures convert exogenous ^{14}C-labeled 18:2(n-6) into labeled products that cochromatograph with authentic standards of 20:3(n-6) and 20:4(n-6). Hence, these epidermal skin cells appear to have the metabolic capability of synthesizing arachidonate when sufficient 18:2(n-6) is supplied.

Rosenthal and Whitehurst (9) have previously demonstrated that cultured human fibroblasts possess Δ^5 and Δ^6

desaturase activity, and can convert exogenous 18:2(n-6) into 20:4(n-6). These fibroblasts are cultivated from the normal residents of the dermal portion of the skin. The current findings indicate that the cultivated residents of the epidermis, keratinocytes, can also actively convert LA into AA. Although the possibility of contamination of the keratinocyte cultures used in these studies with dermal fibroblasts was of initial concern, the demonstration of active conversion of 18:2(n-6) into 20:4(n-6) by human keratinocyte cultures that contain no detectable vimentin-positive fibroblasts make the likelihood of metabolic contributions by fibroblasts quite negligible. The observed conversion represents the products synthesized by the cultivated keratinocytes themselves.

Previous in vivo studies by Chapkin and Ziboh (6) and Chapkin et al. (7) of human, guinea pig, and rat skin, utilizing microsomal preparations of predominantly epidermal slices have, however, demonstrated an inability of this tissue to convert exogenous 18:2(n-6) into 20:4(n-6). One possible explanation for the different findings in the current study is the age of the animals used. The current study utilized cultures derived from newborn mouse or human skin, while the previous investigations (6, 7) utilized adult skin. Indeed, age-related differences in desaturation have been reported (26).

However, a second possible explanation for the ability of cultured cells and not the microsomal preparations derived from intact epidermal tissue to convert 18:2(n-6) into 20:4(n-6) is that the tissue culture system itself may induce these enzymatic activities. There are, indeed, many obvious differences between the in vitro and in vivo systems, the most notable of which may be the widespread use of fetal calf serum (FCS) rather than species-specific sera in the cultivation medium of routine tissue culture systems. Additionally, FCS is generally used in final concentrations of 5–10%, resulting in significant reduction in the levels of hormone mediators. Furthermore, and of particular interest to these studies, is the relative deficiency of FCS in LA when compared to mouse serum (21) or horse, swine, or bovine

TABLE 1. Fatty acid composition[a] of mouse keratinocyte cultures supplemented with 75 μM of either 18:3(n-6) or 20:(n-6)

	18-hr Incubation			64-hr Incubation		
	Control (10)[b]	18:3 (6)[c]	20:2 (6)[d]	Control (6)[b]	18:3 (4)[c]	20:2 (4)[d]
18:2(n-6)	16.24 ± 0.74	15.36 ± 0.75	18.46 ± 0.58**	9.81 ± 0.32	8.62 ± 0.59**	15.13 ± 1.3*
18:3(n-6	0.18 ± 0.33	4.72 ± 1.2*	0.23 ± 0.34	0.05 ± 0.1	5.59 ± 2.6*	0.17 ± 0.20
20:2(n-6)	0.32 ± 0.42	0.31 ± 0.48	7.36 ± 2.0*	0.28 ± 0.22	0.59 ± 0.41	12.58 ± 4.1**
20:3(n-6)	1.68 ± 0.38	4.35 ± 0.38*	1.09 ± 0.12**	1.78 ± 0.12	7.61 ± 0.33*	1.56 ± 0.24
20:4(n-6)	5.97 ± 0.50	5.52 ± 0.58	4.07 ± 0.13*	6.05 ± 0.15	8.82 ± 0.27*	5.18 ± 0.49**

[a]Expressed as means ± SD of the percent of FAME present in the total cellular lipid extracts. Number in parenthesis = n; Only the fatty acids of interest are included, therefore total does not equal 100%.
[b]Control cultures incubated in DMEM + 10% FCS.
[c]Cultures incubated in DMEM + 10% FCS + 75 μM 18:3(n-6).
[d]Cultures incubated in DMEM + 10% FCS + 75 μM 20:2(n-6).
*Significantly different from control, P value ≤ 0.005 by Student's t test (paired, two-tailed).
**Significantly different from control P value ≤ 0.05 by Student's t test (paired, two-tailed).

calf serum (27). As a result, the cells cultivated in this FCS-containing medium exhibit a progressive decrease in LA content. Hence, these cultivated cells may be considered functionally EFA-deficient. Since EFA deficiency is well-known to induce enzymatic activities in vivo, such as increases in Δ^6 activity noted in the livers of EFA-deficient animals (2, 28–30), it is not unreasonable to suggest that increased desaturase activity is similarly induced in the relatively EFA-deficient tissue culture system. Work by Rosenthal and Whitehurst has alluded to this possibility: fibroblasts cultivated in delipidized serum demonstrate induction of Δ^6 desaturase activity (9), as do endothelial cells cultivated in medium containing lowered concentrations of FCS (31). Although normal FCS is not delipidized, the levels of linoleic acid in tissue culture medium containing 10% FCS is 50 times lower than that found in the in vivo situation, and a relative EFA deficiency may develop in the cells cultivated for long periods of time in FCS-containing medium. This relative EFA deficiency may account for the observed differences in the metabolic capability of the in vivo tissue and the cultured cells.

A third explanation may lie in the fact that the previously reported desaturation studies utilizing microsomal preparation depend on the conversion of the fatty acid precursor into the activated fatty acyl CoA thioester. This enzymatic activity may be more active in the intact cultured preparations used in the present study than in the epidermal microsomal preparations used by others (6, 7). Our current investigations are aimed at distinguishing between these three possibilities.

The pattern of accumulation of radiolabeled product in the cultivated keratinocytes along with the supplementation studies utilizing non-radiolabeled 18:(n-6) or 20:2(n-6) suggest that the preferred pathway for conversion of linoleate into arachidonate is the classical one, in which the Δ^6 desaturase constitutes the first step (Fig. 6). An optional pathway, in which elongation of 18:2(n-6) to 20:2(n-6) constitutes the first step, although controversial in liver (24, 32), has been described in other epithelial tissues such as human testes (33), colon, and bladder (23). The current study suggests that this pathway is probably not the preferred one in cultivated keratinocytes. To definitively exclude the operation of the "optional" pathway, more specific metabolic studies utilizing the specifically radiolabeled precursor 20:2(n-6) (11, 14) should be done. At this point however, our data suggest that the major route of synthesis of arachidonic acid is via the classically described pathway.

This study also serves as a cautionary note: although tissue culture systems provide a good tool for the analysis of metabolic pathways, the resultant information should be carefully assessed. Since the tissue culture system often utilizes FA concentrations that differ markedly from the in vivo state, subsequent alteration of cellular FA content and the possible effects on metabolic pathways should be investi-

gated. Although cultured murine and human keratinocytes can convert exogenously supplied 18:2(n-6) into 20:4(n-6), it remains to be determined whether or not the murine or human epidermis, either in normal or diseased conditions, can do the same. ▪

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