Fatty acid oxygenase activity of human hair roots

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Abstract The extent to which fatty acid oxygenases are activated in the normal epidermis is not known. Characterization of the regio- and stereospecificity of the monohydroxylated derivatives of arachidonic and linoleic acid produced by human hair roots is needed to define the enzymatic origin of these compounds and to define a possible role for fatty acid oxygenases in growth, differentiation, and pathology of human hair. Hair roots epilated from normal human volunteers were incubated with radiolabeled arachidonic acid or linoleic acid and the monohydroxylated derivatives produced in vitro were characterized. Incubation of hair roots with [^{14}C]arachidonic acid resulted in the production of 15(S)-[^{14}C]hydroxyeicosatetraenoic acid and 12(S,R)-[^{14}C]hydroxyeicosatetraenoic acid (mean S/R ratio, 2.5). 13(S)-[^{14}C]hydroxyoctadecadienoic acid was the principal product of incubations with [^{14}C]linoleic acid. No radiolabeled products were derived from incubations with heat-denatured hair roots. The fatty acid oxygenase activity of anagen hair roots was inhibited by nordihydroguaiaretic acid and was greatest in the hair root bulb. ▩ The strict S-stereospecificity and the regiospecificity of the n–6 oxygenase are strong evidence for the presence of a 15-lipoxygenase in human hair roots, similar to that identified in cultured human keratinocytes. The stereospecificity of the 12-HETE produced by human hair roots is not compatible with the sole action of 12-lipoxygenase.—Baer, A. N., and F. A. Green. Fatty acid oxygenase activity of human hair roots. *J. Lipid Res.* 1993. **34:** 1505–1514.

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The physiologic and pathologic roles of fatty acid oxygenases in the human epidermis have yet to be defined but there are strong indications that such roles may be important. Cultured human keratinocytes selectively express 15-lipoxygenase activity (1–3), which converts arachidonic acid to 15-hydroxyeicosatetraenoic acid (15-HETE) and linoleic acid to 13-hydroxyoctadecadienoic acid (13-HODE). Both 12-hydroxyeicosatetraenoic acid (12-HETE) and 15-HETE are produced when suspensions or homogenates of freshly isolated epidermal cells are incubated with arachidonic acid (4–6). Terminally differentiated buccal epithelial cells, obtained from the human oral cavity, metabolize exogenous arachidonic acid to yield predominantly 12-HETE (7). The extent to which the fatty acid oxygenases in the epidermis are activated in vivo is not known. In an analysis of skin surface lipids extracted from the hair and foreheads of normal subjects, the n–6 hydroxylated derivative of linoleic acid, 13-HODE, was found to be present as an enantiomeric mixture, with an average S/R ratio of 2.2, and in amounts twice that of its regioisomer, 9-HODE (8). These observations constitute evidence for the presence of an n–6 oxygenase in the normal epidermis which may be activated in vivo under physiologic conditions.

The fatty acid oxygenase activities in the epidermis have been attributed variously to cyclooxygenase, lipoxygenase, or a cytochrome P450 monooxygenase (2, 4, 9, 10). Certain hydroxylated fatty acid derivatives may have their origin in the epidermis from more than one type of oxygenase as well as from nonenzymatic pathways. One example is 12-HETE which is found in high concentrations in psoriatic epidermis (11). In extracts of psoriatic skin scales, the 12-HETE is almost exclusively the R-stereoisomer (12). In contrast, the 12-HETE that is produced ex vivo by suspensions of human epidermal cells is an enantiomeric mixture, with a predominance of the S-stereoisomer (4). The 12-HETE produced ex vivo by buccal epithelial cells is strictly the S-stereoisomer, consistent with its production by 12-lipoxygenase (7). These findings suggest that more than one arachidonic acid 12-oxygenase is expressed in the epidermis, possibly as a function of epidermal differentiation and/or inflammation.

The role of the fatty acid oxygenases in the physiology of the normal epidermis is not known. While cyclooxygenase and cytochrome P450 activities are nearly ubiquitous in organ systems, lipoxygenase activity appears to be restricted to a small number of cell types, including hematopoietic cells, keratinocytes, pancreatic islet cells,

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and tracheal epithelial cells (1, 2, 13, 14). The activation of lipoxygenases during acute inflammatory and allergic responses has been well documented (15). There are only a few examples where lipoxygenase activity appears to have a role in normal cellular physiology. One example is that of the reticulocyte 15-lipoxygenase which may participate in the programmed degradation of mitochondria within the reticulocyte, a process essential for the maturation of the reticulocyte to the erythrocyte (16, 17). Whether keratinocyte 15-lipoxygenase might be involved in the differentiation program of the epidermis and of its appendages is an important question.

We report herein a characterization of the mono-hydroxylated derivatives of arachidonic acid and of linoleic acid produced in vitro by freshly plucked human hair roots. Evidence pointing to a possible role for the fatty acid oxygenases in the growth and differentiation of human hair was also sought.

MATERIALS AND METHODS

Reagents

[5,6,8,9,11,12,14,15-³H]arachidonic acid (210 Ci/mmol), [1-¹⁴C]arachidonic acid (58.3 mCi/mmol), [1-¹⁴C]linoleic acid (54 mCi/mmol), and 12(S)[5,6,8,9,11,12,14,15-³H(N)]hydroxy-5,8,10,14-eicosatetraenoic acid (172 Ci/mmol) were purchased from Amersham (Arlington Heights, IL). 15(S)[5,6,8,9,11,12,14,15-³H(N)]hydroxy-5,8,11,13-eicosatetraenoic acid (187 Ci/mmol) was purchased from New England Nuclear Research Products (DuPont Company, Boston, MA). Racemic 15 [1-¹⁴C]HETE and racemic 12 [1-¹⁴C]HETE standards were generated from [1-¹⁴C]arachidonic acid in a reaction with hydrogen peroxide and cupric ions (18) to yield the hydroperoxy compounds which were then reduced with sodium borohydride. Racemic 13 [1-¹⁴C]hydroxyocta-9Z,11E-dienoic acid and racemic 9-[1-¹⁴C]hydroxyocta-10E,12Z-dienoic acid were prepared by air autooxidation of [1-¹⁴C]linoleic acid (54 mCi/mmol). These racemic derivatives of [1-¹⁴C]arachidonic and linoleic acids were purified with reversed- and straight-phase HPLC before use. Unlabeled 13(S)-HODE, 15(S)-HETE, and 12(S)-HETE standards were purchased from Biomol Research Laboratories (Plymouth Meeting, PA). Racemic 13-HODE and racemic 9-HODE standards were purchased from Cayman Chemical Co. (Ann Arbor, MI). The methyl esters of these arachidonic acid and linoleic acid derivatives were prepared using ethereal diazomethane. All solvents were of HPLC grade.

Human material

Scalp, beard, and eyebrow hair were plucked from normal adult male and female volunteers, either singly with the aid of tweezers or multiply with the aid of surgical needle-holding forceps, as described by Crounse and Brown (19). After excision of the hair shaft, the hair roots were placed in a Petri dish containing Dulbecco's phosphate-buffered saline. With the aid of a dissecting microscope, anagen (representing hair roots in an active growth phase) and telogen (resting phase) hair roots were distinguished on the basis of characteristic morphologic features (20, 21). The morphologic features of anagen hair roots were identified by staining with 4-dimethyl-aminocinnamaldehyde (21). In the hair plucked from the scalp, the anagen hair roots had an intact inner and outer root sheath and a pigmented bulbous end. The scalp telogen hair roots had a terminal epithelial sac but lacked an inner or outer root sheath. The anagen hair roots plucked from the beard often had an altered morphology, with an absent or fragmented outer and inner root sheath, a phenomenon which has been attributed to the method of epilation (19, 20). In some experiments, scalp anagen hair roots were cut transversely with a scalpel in order to yield three sequential sections of hair root. These included, from proximal to distal, the bulb of the hair root (representing no more than 25% of the length of the hair root circumscribed by the inner and outer root sheaths), the remaining length of hair root circumscribed by the inner and outer root sheaths, and a section of adjacent hair shaft equivalent in length to the distance defined by the inner and outer root sheaths.

The hair roots or sections thereof (8–40/tube) were placed in 0.5 ml of medium 199 with 40 mM HEPES buffer (pH 7.6) and incubated with 2 μCi (9.5 pmol) of [5,6,8,9,11,12,14,15-³H]arachidonic acid, 2 μCi (34 nmol) of [1-¹⁴C]arachidonic acid, or 2 μCi (37 nmol) of [1-¹⁴C]linoleic acid for 115–180 min in a 37°C water bath. Each radiolabeled substrate was used carrier-free in order to maximize radioactive product formation. As a control, suspensions of an equal number of hair roots were immersed in a boiling water bath for 15 min prior to the incubation with radiolabeled fatty acid substrate. In some experiments, the anagen hair roots, suspended in 0.5–1.0 ml of medium, were sonicated at the microtip limit with four bursts of 10 sec each (Branson Instruments Co., Plainview, NY). The resulting suspension of released cells and cell fragments was then incubated either alone or with the residual hair shafts in the presence of radiolabeled fatty acid substrate as described above. In order to assess the effects of enzyme inhibitors, a suspension of hair follicle cells and cell fragments, released after sonication of anagen hair roots, was divided into equal aliquots. Each aliquot was then pre-incubated with the inhibitor (added in a vehicle of dimethylsulfoxide, 1 λ/ml) or vehicle alone for 15 min at 37°C prior to the addition of radiolabeled substrate. The protein content of these aliquots was measured using the method of Lowry et al. (22). In order to test for inhibition of oxygenase activity by exposure to carbon monoxide, aliquots of hair follicle cells and cell fragments released from hair shafts by sonication

were suspended in 250 μl air-saturated medium to which was added 750 μl medium gassed for 15 min with carbon monoxide or with nitrogen for the control. The incubations with radiolabeled substrate were then carried out in an atmosphere of room air.

At the end of the incubation period, the tubes were snap-frozen and then lyophilized. The lyophilized material was then extracted with methanol–chloroform 1:1 followed by water (0.8 vol) and 2.0 M ammonium formate, pH 3.2 (0.1 vol) (23). Unlabeled 15-HETE was added as an internal standard to monitor product recovery. The lower organic layer was washed twice with pure upper phase solvents and then dried under vacuum. The average recovery with this extraction procedure was 80 ± 2% (mean ± SEM). The extracted lipids were reconstituted into a mobile phase consisting of methanol–water–acetic acid 80:20:1 for the separation of the monohydroxylated derivatives of arachidonic acid by reversed-phase HPLC. In some analyses, the lipids extracted from incubations with [^{14}C]linoleic acid were methylated with ethereal diazomethane prior to straight-phase HPLC using a mobile phase consisting of hexane–isopropanol 1000:15.

Reversed-phase HPLC

Reversed-phase HPLC was performed isocratically on a Hewlett-Packard 1090 liquid chromatograph (LC) using Hewlett-Packard 5 μM ODS-Hypersil columns 200 × 4.6 mm in length at a flow rate of 0.4 ml/min. This instrument is equipped with a diode array spectrophotometer and a computer for on-line display and storage of ultraviolet spectra and absorbance data. The LC runs were monitored at a wavelength of 236 nm. A Radiomatic Flo-One A-140 (Radiomatic Instruments and Chemical Co., Inc., Tampa, FL) radioactivity detector was used for concurrent measurement of ^{3}H or ^{14}C in the outflow from the HPLC spectrophotometer. An interface between the radioactivity detector and the Hewlett-Packard Chem-Station computer permitted storage of the radioactivity data for subsequent analysis. Radioactivity peaks were quantified by the use of existing software programs in the Hewlett-Packard Chem-Station computer for peak integration. The total radioactivity in specific peaks on reversed-phase HPLC runs was calculated using a standard curve generated by successive injections of defined amounts of 15(S)[^{3}H]HETE standard. All chromatograms shown in the figures are reproduced directly from the HPLC computer plots.

Straight-phase HPLC

Straight-phase HPLC was performed isocratically on the same LC instrument using a Microsorb 5 μM SI column (Rainin Instrument Co., Woburn, MA), 4.6 mm × 250 mm in length, at a flow rate of 0.4 ml/min.

Stereochemical analyses

In preliminary studies, it was determined that the elution of the ^{14}C-labeled and unlabeled fatty acid derivatives coincide during reversed- and straight-phase chromatography. Accordingly, unlabeled 15-HETE, 12-HETE, 13-HODE, or 9-HODE was added to the extracted samples in order to allow monitoring of the elution of the radioactive compound of interest from both the reversed-phase and straight-phase columns. During reversed-phase HPLC of the extracts from the hair root incubations with [^{14}C]arachidonic acid, the LC eluate was collected during the elution of the 15-HETE and the 12-HETE standards. The compounds within appropriate peaks were subsequently methylated with ethereal diazomethane and then rechromatographed on a straight-phase Microsorb SI column using hexane–isopropanol 1000:4 as the mobile phase. During straight-phase HPLC of the methylated extracts from the hair root incubations with [^{14}C]linoleic acid, the LC eluate was collected during the elution of 13-[^{14}C]HODE methyl ester and during that of 9-[^{14}C]HODE methyl ester. The methylated arachidonic acid or linoleic acid derivatives, collected during the straight-phase LC runs, were then rechromatographed on two 4.6 × 250 mm Bakerbond straight-phase chiral LC columns in series ((R)-N-3,5-dinitrobenzoylphenyl glycine residues covalently bonded to silica; J. T. Baker Inc., Phillipsburg, NJ). The mobile phase for this straight-phase HPLC consisted of hexane–isopropanol 1000:4. The eluate from the chiral HPLC columns was monitored for both radioactivity and UV absorbance. The R and S assignments were established using HETE and HODE standards of known chirality. The identity and chirality of the radioactive compounds were confirmed in all cases by performing mixing experiments in which radioactive synthetic methyl ester standards were added to the derivatized material originally obtained from the hair root incubations.

RESULTS

The chromatographic separation of extracts from incubations of plucked human hair roots with [^{3}H]arachidonic acid yielded two major radioactive peaks. One eluted at 22.5 min and had a retention time identical to that of authentic 15[^{3}H]HETE standard. The second eluted at 25 min and had a retention time identical to that of authentic 12-[^{3}H]HETE standard (**Fig. 1**). These peaks were not observed in radiochromatograms of extracts derived from incubations in which the hair roots were first heat-denatured (Fig. 1). In five incubations of scalp anagen hair roots obtained from five different individuals (four male, one female), the ratio of the production of
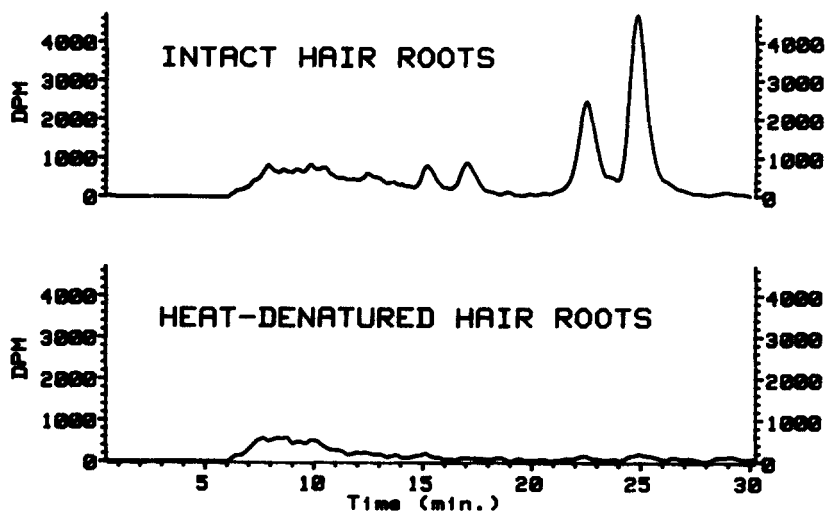
15-HETE to that of 12-HETE averaged 0.8 ± 0.2 (mean ± SEM), with the amount of 15-HETE exceeding that of 12-HETE in two of the five samples. When the extracts of hair root incubations with [³H]arachidonic acid were treated with ethereal diazomethane prior to reversed-phase HPLC, the retention times of the two major radioactive peaks were each delayed by approximately 15 min and coincided with the retention times of the methyl ester derivatives of 15-[³H]HETE and 12-[³H]HETE (data not shown). The time course for the production of 15-[³H]HETE and 12-[³H]HETE from [³H]arachidonic acid by beard anagen hair roots is shown in **Fig. 2**. Maximum HETE production was observed within the first 120 min of incubation.

Extracts from incubation of hair roots with [¹⁴C]linoleic acid were methylated prior to straight-phase chromatography, using conditions that resolve the methyl ester derivatives of the two major products of linoleic acid, 13-HODE and 9-HODE. A single major radioactive peak was seen in each radiochromatogram and this major peak shared the retention time (20.5 min) of the 13-[¹⁴C]HODE methyl ester standard (**Fig. 3**). A much smaller peak at the retention time of 9-[¹⁴C]HODE methyl ester standard (23.8 min) was also evident. No radioactive peaks were seen in extracts of incubations of [¹⁴C]linoleic acid with heat-denatured hair roots (Fig. 3).

The stereospecificity of the 15-[¹⁴C]HETE and 12-[¹⁴C]HETE generated during the incubation of the plucked hair roots with [¹⁴C]arachidonic acid was analyzed using chiral HPLC columns under straight-phase conditions. For these analyses, the radioactive compounds were collected during reversed-phase chromatography and treated with ethereal diazomethane. The resulting methyl esters were re-chromatographed using straight-phase conditions that resolve 15-HETE from 12-HETE. An aliquot of this collected material was then chromatographed on straight-phase chiral columns and the eluate

was monitored for radioactivity. Mixing experiments were performed with racemic 15-[¹⁴C]HETE and 12-[¹⁴C]HETE methyl ester standards in order to confirm the identity of each methyl ester and to differentiate the stereoisomers. Chromatographic conditions on the chiral-phase columns were used which resolved these two radioactive compounds and their stereoisomers (**Fig. 4**). In each of three analyses, the 15-[¹⁴C]HETE produced in vitro by the hair roots eluted from chiral-phase columns as a single peak (**Fig. 5**). Racemic 15-[¹⁴C]HETE methyl
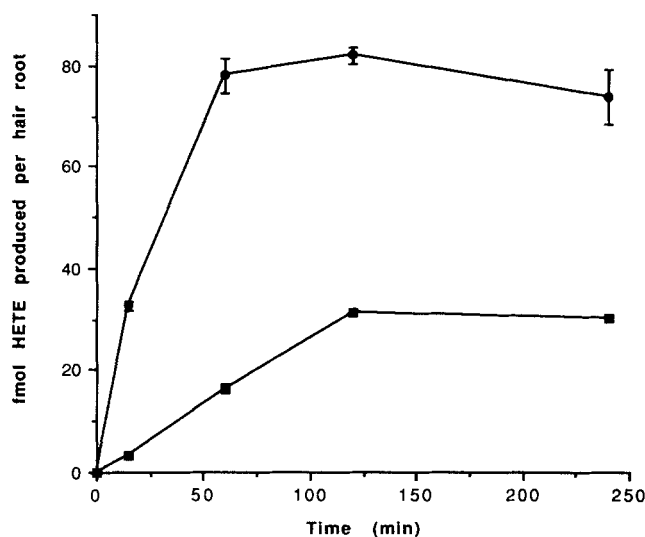

Fig. 2. Time course for the production of 15-[³H]HETE (solid squares) and 12-[³H]HETE (solid circles) from [³H]arachidonic acid by anagen hair roots. Twenty-six beard anagen hair roots, suspended in 1 ml medium, were incubated at 37°C in the presence of 4 μCi [³H]arachidonic acid. Triplicate aliquots of 50 μl each were taken at the time points shown and the amounts of 15-[³H]HETE and 12-[³H]HETE were measured in each using reversed-phase HPLC with radioactive monitoring of the eluate. Standard error bars are drawn for each time point. The results shown here are representative of the three time course experiments that were performed.

Fig. 3. Straight-phase HPLC radiochromatograms of the radiolabeled products of the incubation of [^{14}C]linoleic acid with either 10 intact (upper chromatogram) or 10 heat-denatured (lower chromatogram) anagen hair roots epilated from the scalp of a normal male volunteer. The extracts of these incubations were methylated prior to the straight-phase chromatography shown here. In the chromatogram of the products generated by the intact hair roots, the major peak of radioactivity had the retention time (20.5 min) of authentic 13-[^{14}C]HODE methyl ester standard. A small radioactive peak eluting at 23.8 min had the retention time of authentic 9-[^{14}C]HODE methyl ester standard. No significant radiolabeled products were generated by the heat-denatured hair roots.
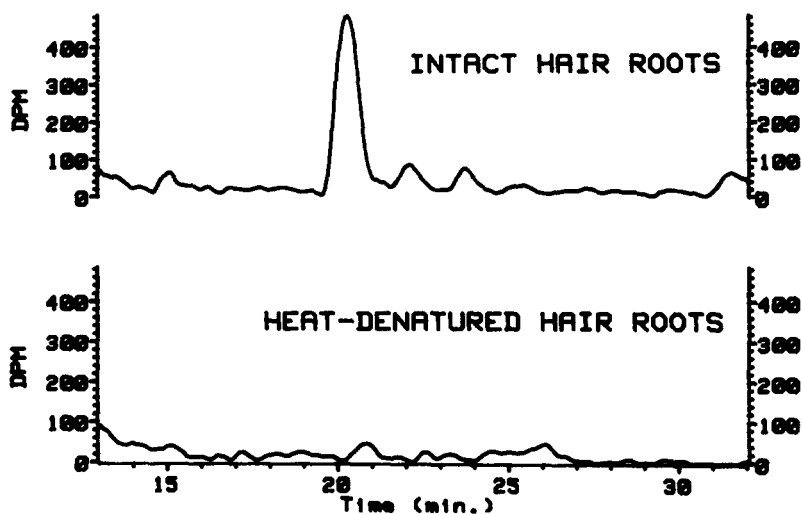

Fig. 4. Straight-phase chiral HPLC radiochromatogram of the racemic 12-[^{14}C]HETE and 15-[^{14}C]HETE methyl ester standards. With these chromatographic conditions, 12-HETE methyl ester stereoisomers elute prior to those of 15-HETE methyl ester and the S-stereoisomers elute first. These two racemic methyl ester standards and their respective stereoisomers were adequately resolved using a mobile phase consisting of hexane–isopropanol 1000:4 and a flow rate of 0.4 ml/min.
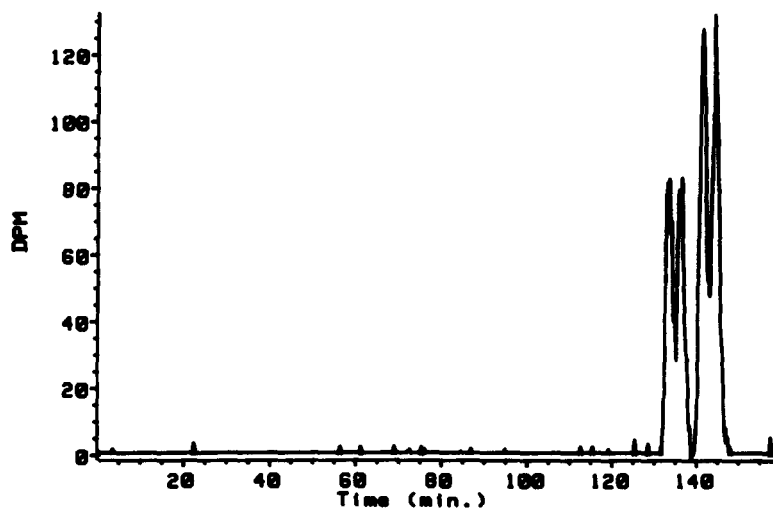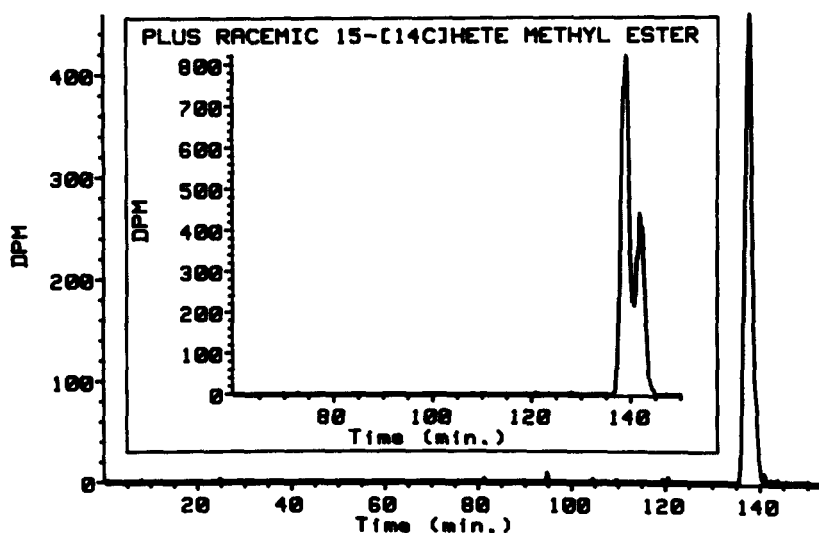
Fig. 5. Representative straight-phase chiral HPLC radiochromatograms of the methyl ester of the radiolabeled 15-[^{14}C]HETE produced in the incubation of [^{14}C]arachidonic acid with hair roots epilated from the beard of a normal male volunteer. The outer chromatogram is that of material eluting at 22.5 min on reversed-phase HPLC (as in Fig. 1) that was collected, methylated, and re-chromatographed on straight-phase columns prior to the chiral-phase chromatography shown here. The chromatogram shown in the inset is that of an equal aliquot of the same material to which racemic 15-[^{14}C]HETE methyl ester standard had been added. The experimentally produced material co-eluted with the S-stereoisomer of the racemic 15-[^{14}C]HETE methyl ester standard.
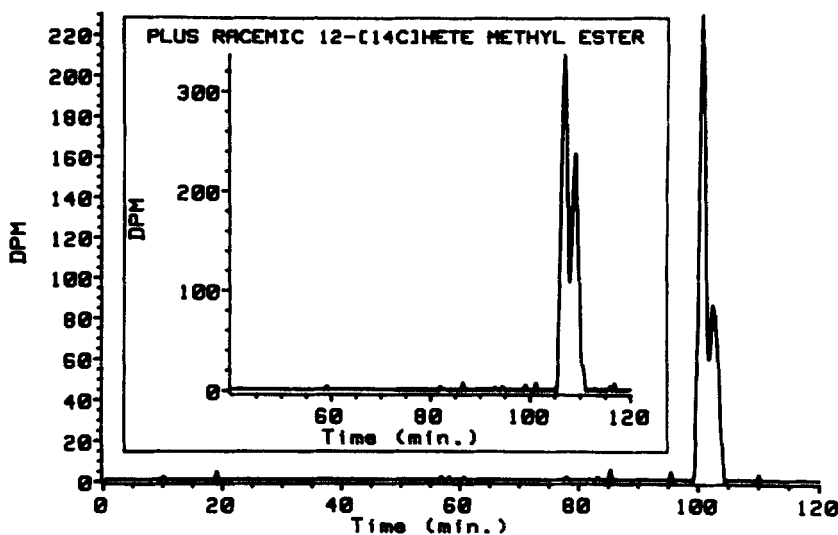
Fig. 6. Representative straight-phase chiral HPLC radiochromatograms of the methyl ester of the radio-labeled 12-[^{14}C]HETE produced in the incubation of [^{14}C]arachidonic acid with hair roots epilated from the beard of a normal male. The outer chromatogram is that of material eluting at 25 min on reversed-phase HPLC (as in Fig. 1) that was collected, methylated, and re-chromatographed on straight-phase columns prior to the chiral-phase chromatography shown here. The chromatogram shown in the inset is that of an equal aliquot of the same material to which racemic 12-[^{14}C]HETE methyl ester standard had been added. The S/R ratio of the experimentally produced material was 2.2 and this ratio decreased, as expected, following the addition of racemic 12-[^{14}C]HETE methyl ester standard.

ester standard was added to aliquots of the methyl esters of the in vitro-produced 15-[^{14}C]HETE. On repeat chiral-phase chromatography of the resultant mixture, it was apparent that the in vitro-produced material co-eluted with the first eluting peak, representing the S-stereoisomer of 15-HETE (Fig. 5, inset). In each of three analyses, the 12-[^{14}C]HETE produced in vitro by the hair roots eluted from chiral-phase columns as an enantiomeric mixture (**Fig. 6**), with the S/R ratio averaging 2.5 ± 0.2 (mean ± SEM). Chiral-phase chromatography of a mixture of the methyl esters of racemic 12-[^{14}C]HETE standard and in vitro-produced 12-[^{14}C]HETE confirmed the stereochemical designation (Fig. 6, inset).

The 13-[^{14}C]HODE and 9-[^{14}C]HODE produced in vitro during the incubation of [^{14}C]linoleic acid with plucked hair roots were methylated and chromatographed, first on straight-phase columns as a preparative step, and then on chiral-phase columns for analysis. The principal product was 13(S)-[^{14}C]HODE; a small amount of racemic 9-[^{14}C]HODE was also evident (**Fig. 7**). In two analyses, the average ratio of the methyl ester derivative of 13-HODE to that of 9-HODE was 18:1.

The in vitro production of 15-[^{3}H]HETE and of 12-[^{3}H]HETE was compared in six experiments using scalp hair from four different donors; in each, the incubations contained equal numbers of scalp anagen and telogen hair roots. Radiochromatograms from one such experiment are shown in **Fig. 8.** The production of 15-[^{3}H]HETE was 4.9 ± 2.2 (mean ± SEM) times greater and that of 12-[^{3}H]HETE was 4.0 ± 1.8 times greater in the anagen hair roots than in the telogen hair roots. Differences in the relative production of the HETEs by the anagen and the telogen hair roots, evident in Fig. 8, were not a consistent finding. In order to localize the fatty acid oxygenase activities in the anagen hair roots, the roots were sectioned transversely to yield three consecu-
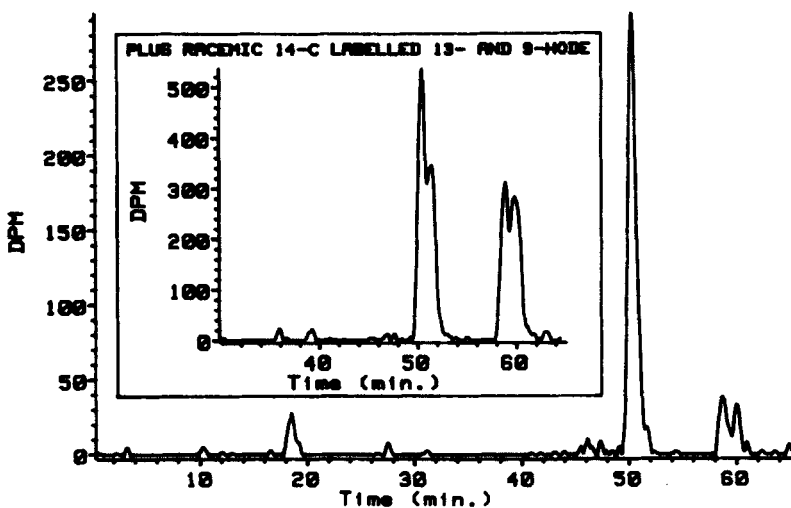

Fig. 7. Representative straight-phase chiral HPLC radiochromatograms of the methyl esters of the radio-labeled 13-[^{14}C]HODE and 9-[^{14}C]HODE produced in the incubation of [^{14}C]linoleic acid with hair roots epilated from the scalp of a normal male. The outer chromatogram is that of the material in the peak at 20.5 min (Fig. 2, upper chromatogram) which was then subjected to the chiral-phase chromatography shown here. The methyl ester of 13-HODE elutes prior to that of 9-HODE and the S-stereoisomer of each compound is the first to elute under these chromatographic conditions. The chromatogram shown in the inset is that of an equal aliquot of the same material to which racemic 13-[^{14}C]HODE and 9-[^{14}C]HODE methyl ester standards had been added. The mixing experiment demonstrates that the principal product of the incubation of linoleic acid with hair roots is 13(S)-HODE, with racemic 9-HODE constituting a minor product.

Fig. 8. Reversed-phase HPLC radiochromatograms of the radiolabeled products of the incubation of [³H]arachidonic acid with anagen (left panel) and telogen (right panel) hair roots from the scalp of a normal male. The production of 15- and 12-HETE was consistently higher in the anagen hair roots. However, the difference in the relative production of the two HETEs by the anagen and telogen hair roots, observed in this experiment, was not a consistent finding.
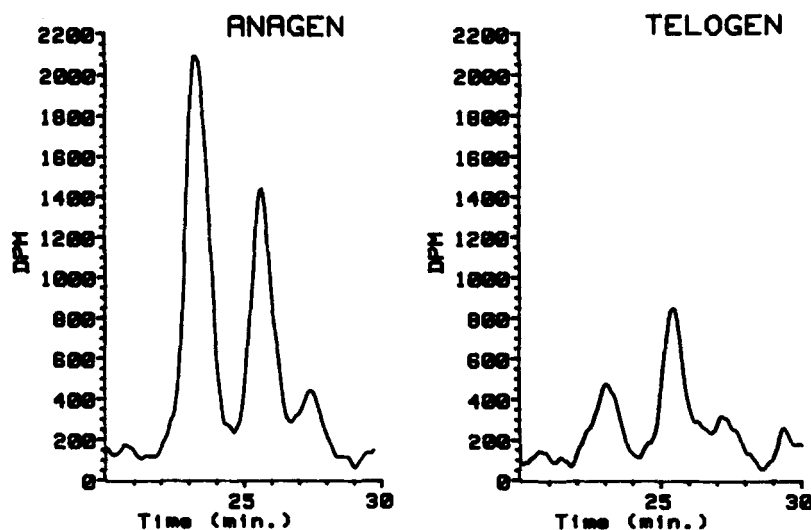
tive segments: the proximal root bulb which contains the germinative matrix, a larger middle segment which contains the remaining length of outer root sheath, and a section of distal hair shaft equal in length to that defined by the outer and inner root sheaths. In five experiments using scalp hair from two different donors, the production of 15-[³H]HETE and of 12-[³H]HETE was compared in incubations containing equal numbers of these consecutive transverse sections of anagen hair roots. The radiochromatograms in **Fig. 9** are from one such experiment, in which the metabolism of [³H]arachidonic acid by the hair root bulb was compared to that of the adjacent segment which contained the remaining length of outer root sheath. The production of 15-[³H]HETE was 3.3 ± 1.0 times greater and that of 12-[³H]HETE was 1.7 ± 0.5 times greater in the root bulb as compared to this adjacent "sheath" segment, even though the section of root

bulb was approximately 25% of the length of the proximal root bulb segment. Similarly, the production of 15-[³H]HETE was 4.2 ± 1.5 times greater and that of 12-[³H]HETE was 2.1 ± 0.5 times greater in the proximal root bulb segment as compared to the most distal segment which consisted of hair shaft alone.

In order to examine the effect of enzyme inhibitors on HETE production by the hair roots in vitro, several techniques were evaluated for freeing cells from the hair roots in order to produce a homogeneous suspension which could then be aliquoted equally for inhibition studies. It was observed that sonication of anagen hair roots resulted in the release of cells and cell fragments as well as a significant augmentation of enzyme activity. Thus, suspensions of scalp anagen hair roots that had been sonicated yielded 5.2 times more 15-HETE and 14.7 times more 12-HETE than suspensions containing an equal

Fig. 9. Reversed-phase HPLC radiochromatograms of the radiolabeled products of the incubations of [³H]arachidonic acid with either 14 segments of scalp hair root bulb containing the germinative matrix (upper chromatogram) or 14 adjacent segments defined by remaining length of the outer root sheath (lower chromatogram).
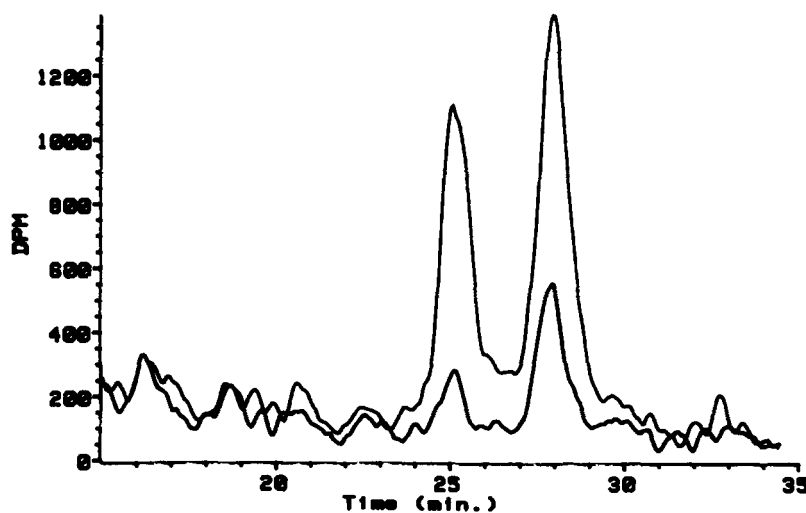
TABLE 1. Effects of inhibitors on oxygenase activities in hair root cells

Experiment	Inhibitor	Radioactive Product Formation	
		15-[³H]HETE	12-[³H]HETE
		fmol/mg protein/h	
1	Exogenous substrate alone	15.4 ± 0.3	66.3 ± 2.3
	+ Indomethacin (10 μM)	16.5 ± 0.3	66.5 ± 1.2
	+ Nordihydroguaiaretic acid (30 μM)	4.1 ± 0.2	5.9 ± 0.3
2	Exogenous substrate alone	53.5 ± 1.7	200.3 ± 14.4
	+ SKF 525 A (500 μM)	44.1 ± 1.6	209.4 ± 20.3
3	Nitrogen-saturated buffer (control)	31.0 ± 3.2	131.2 ± 5.3
	Carbon monoxide-saturated buffer	28.4 ± 2.5	142.4 ± 19.0

Values shown are the means ± SEM of triplicate determinations in each experiment. The N_2:O_2 molar ratio was 14:1 and the CO:O_2 molar ratio was 11:1.

number of hair roots that had not been sonicated (mean of two experiments). The metabolic activity in these sonicated hair root suspensions was predominantly in the cells released from the hair roots (i.e., the sonicate) and not in the residual hair shafts.

The effects of enzyme inhibitors on the metabolism of [³H]arachidonic acid by equal aliquots of anagen hair root sonicates are shown in **Table 1**. The production of 15-[³H]HETE and 12-[³H]HETE by the hair roots was inhibited 73% and 91%, respectively, by nordihydroguaiaretic acid (30 μM), but no significant inhibition of either oxygenase activity was observed with indomethacin, SKF 525A, or carbon monoxide.

DISCUSSION

The incubation of freshly plucked human hair roots with radiolabeled arachidonic acid resulted in the production of the monohydroxylated derivatives, 15-HETE and 12-HETE. These results confirm and extend those reported previously by Henneicke-von Zepelin et al. (24). Among samples of hair roots obtained from different adult volunteers, there was marked variation in the relative production of the two arachidonic acid products, an observation which suggests that 15-HETE and 12-HETE are the products of two separate enzyme activities.

With chiral-phase HPLC analysis, it was found that the in vitro-produced 15-HETE demonstrated strict S-stereospecificity. Mammalian lipoxygenases have been found uniformly to insert molecular oxygen with strict S-stereospecificity at the chiral center of the 1,4-*cis, cis*-pentadiene moiety which is common to both arachidonic acid and linoleic acid (25). This strict S-stereospecificity is not a characteristic of the other two fatty acid oxygenases that can catalyze the monohydroxylation of arachidonic acid, namely cyclooxygenase and the cytochrome P450 monooxygenases (26, 27). The stereochemical findings in

the current studies constitute a strong argument for the designation of the n−6 oxygenase in human hair roots as 15-lipoxygenase. Additional evidence for the presence of a 15-lipoxygenase in the human hair roots was provided by the analysis of the products of the incubation of radiolabeled linoleic acid with the hair roots. The principal product was 13(S)-HODE; small amounts of racemic 9-HODE were also found. Both the regiospecificity and the stereospecificity of this reaction are characteristic of the action of a 15-lipoxygenase and would not be seen in reactions catalyzed by either cyclooxygenase or cytochrome P450 monooxygenases (17).

The 12-HETE produced in vitro by the hair roots was an enantiomeric mixture, with an average S/R ratio of 2.5. The stereochemistry of this product cannot be accounted for by the sole action of a 12-lipoxygenase, such as has been described in human buccal epithelial cells. The arachidonic acid 12-oxygenase in hair roots was inhibited by nordihydroguaiaretic acid, but not by indomethacin, SKF 525A, or carbon monoxide. Holtzman, Turk, and Pentland (4) demonstrated that the 12-HETE produced in vitro by isolated human epidermal cells was also an enantiomeric mixture, with S/R ratios ranging from 2:1 to 8:1. The 12-oxygenase that they characterized was membrane-bound, and its activity was augmented by the cofactor, NADPH, and inhibited by carbon monoxide. These observations suggested that epidermal cells contain a cytochrome P450 monooxygenase which can produce 12(S,R)-HETE. Psoriatic skin scales contain 12(R)-HETE and incubation of psoriatic skin scales in vitro with radiolabeled arachidonic acid results in the production of radiolabeled 12-HETE which is predominantly the R-stereoisomer (9, 28). Thus, there appears to be more than one oxygenase in the epidermis capable of producing 12-HETE, and the nature of these oxygenases needs to be defined. It is possible that the 12-HETE produced in vitro by the hair roots is the product of a lipoxygenase and a second oxygenase, such as that observed in psoriatic skin scales.

It has been recognized during the past decade that epidermal cells produce 15-HETE and/or 12-HETE from arachidonic acid and 13-HODE and 9-HODE from linoleic acid (29, 30). These studies have been performed using keratome biopsy specimens, suspensions of freshly isolated epidermal cells, and cultured keratinocytes (1, 2, 4, 31, 32). Localization of specific fatty acid oxygenase activities to subsets of epidermal cells has been sought. Henneicke-von Zepelin et al. (24) have compared the metabolism of [³H]arachidonic acid by basal cells and by suprabasal epidermal cells. In their studies, the production of 15-[³H]HETE predominated in the basal epidermal cells while production of 12-[³H]HETE predominated in the more differentiated suprabasal epidermal cells. These workers did not characterize the chirality of these monohydroxylated derivatives of arachidonic acid. These results from freshly isolated epidermal cells appear to be supported by the observations that 15-HETE is the sole product of incubations of arachidonic acid with cultured human keratinocytes (which are less differentiated on average than those in vivo) while 12-HETE is the primary product in similar incubations with terminally differentiated buccal epithelial cells.

In the current study, in vitro incubation of anagen hair roots with [³H]arachidonic acid resulted in the production of both 15-[³H]HETE and 12-[³H]HETE in ratios that varied widely between donors. This finding differs from that of Henneicke-von Zepelin et al. (24) that 15-HETE production is predominant in hair roots. Methodologic differences may account for this difference, since Henneicke-von Zepelin et al. (24) minced the hair roots prior to study. Their method of epilation was not stated and anagen hair roots were not separated from telogen hair roots. We found the outer root sheath to be frequently absent in anagen roots plucked from the beard. It is possible that the disruption of the outer root sheaths in the beard hair roots, resulting from the method of epilation, may have either exposed less differentiated epidermal cells to the radioactive substrate or have activated 15-lipoxygenase. The increase in oxygenase activity that was observed in the current study after sonication of the hair roots illustrates that mechanical factors, such as the method of epilation or the subsequent handling of the hair roots, can influence the amount of measured oxygenase activity. This increase in activity might occur as a consequence of tissue disruption and release/exposure of more cells to the incubation medium or as a consequence of cell membrane damage, a stimulus which has been shown to activate or "unmask" 15-lipoxygenase in cultured human keratinocytes (1) and in neutrophils (33). In the current study, the extent of monohydroxylation of [³H]arachidonic acid was greater in anagen hair roots as compared to telogen hair roots and in the proximal hair root bulb as compared to adjacent distal segments. These observations suggest that the fatty acid oxygenases of hair roots

are predominantly expressed in the non-keratinized germinative matrix cells of the hair root bulb and/or in the non-keratinized cells of the outer root sheath. It is of note, however, that the relative activity of the two oxygenases did not appear to vary significantly between anagen versus telogen hair roots or between the hair root bulb versus adjacent distal segments. Thus there was no apparent association between regiospecificity and active cell division and growth in the hair root.

There are many factors which could potentially influence the amount and type of oxygenase product in hair roots. These include age and sex of the donor, hair site, pathological states, and method of epilation. Study of these variables may clarify the role of the fatty acid oxygenases in epidermal cell pathophysiology. The previous finding of predominantly 13(S)-HODE in extracts from skin surface lipids is consistent with in vivo activation of 15-lipoxygenase. If this is true, a physiologic role of keratinocyte lipoxygenase activation is possible and awaits definition. Lipoxygenase activation in the epidermis has the potential for affecting cellular function as a result of esterification of free hydroxylated fatty acid derivatives within cellular membranes (9), direct lipoxygenation of esterified membrane fatty acids (34), production of toxic free radical or hydroperoxide intermediates during lipoxygenase activation (35), or the generation of biologically active fatty acid derivatives (36). Future studies of normal and pathological hair root cells may further define the role of these oxygenases. ▮

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