Identification of the major metabolite of 12-HETE produced by renal tubular epithelial cells

Joel A. Gordon,[1] Paul H. Figard, and Arthur A. Spector

Departments of Internal Medicine and Biochemistry, The University of Iowa College of Medicine, Iowa City, IA 52242

Abstract The identification and polarity of release of the major metabolite of 12-HETE produced by cultured canine renal tubular epithelial cells was determined. When incubated with 1.0 μM [^{3}H]12-HETE for 1 h, cultured Madin Darby Canine Kidney (MDCK) cells converted 35% of the radiolabeled 12-HETE to a more polar metabolite. Following high performance liquid chromatography isolation and chemical derivatization, gas–liquid chromatography combined with mass spectrometry was used to identify the compound as 8-hydroxyhexadecatrienoic acid [16:3(8-OH)]. The electron impact mass spectrum of the hydrogenated derivative contained major ions at m/z = 215 and 245, corresponding to cleavage on either side of the trimethylsilyl group, and chemical ionization with NH_3 yielded a major ion at m/z = 359, corresponding to the protonated molecular weight of the methyl ester. Incubation with 25 mM α-naphthoflavone, 20 μM nordihydroguaiaretic acid, and 0.1 mM 4-pentenoic acid failed to inhibit the formation 16:3 (8-OH), suggesting that the formation of 16:3 (8-OH) is not mediated by the cytochrome P-450, lipoxygenase, or mitochondrial β-oxidation pathways. When grown on fibronectin-treated polycarbonate filters, MDCK cells released the 16:3 (8-OH) in both the apical and basolateral directions, irrespective of which side the 12-HETE was encountered. These results demonstrate the conversion of 12-HETE to a 16-carbon monohydroxy derivative by renal tubular epithelium and suggest that this product can be released to either the potential urinary space or the kidney parenchyma and renal microcirculation.—**Gordon, J. A., P. H. Figard, and A. A. Spector.** Identification of the major metabolite of 12-HETE produced by renal tubular epithelial cells. *J. Lipid Res.* 1989. **30:** 731–738.

Supplementary key words 12-HETE • MDCK cells • β-oxidation • 8-hydroxyhexadecatrienoic acid • renal tubular epithelium

12-Hydroxyeicosatetraenoic acid (12-HETE) is a lipoxygenase product formed through the conversion of arachidonic acid to an unstable hydroperoxy derivative, 12-HPETE, followed by rapid reduction to the more stable hydroxylated derivative, 12-HETE (1). 12-HETE is a major product synthesized by activated macrophages and platelets (2, 3). It is incorporated into tissue lipids (4–6) and in addition, has been shown to modulate glucose-stimulated insulin secretion (7) and angiotensin-stimulated aldosterone production (8), stimulate chemotaxis and chemokinesis in neutrophils (9, 10), act as a mitogen for bovine aortic endothelial cells through inhibition of diacylglycerol kinase (11), and inhibit prostaglandin formation in bovine aortic endothelial cells and renal tubular epithelial cells (12, 13).

We have previously described the uptake, distribution, and metabolism of 12-HETE by Madin Darby canine kidney (MDCK) cells (13). When grown on micropore filters, 12-HETE uptake was greater from the basal surface when compared to the apical surface, suggesting that the tubular epithelium may function to remove any 12-HETE released into the renal parenchyma during inflammatory processes. Although 12-HETE was incorporated into cell lipids without modification, it was also converted to several polar and nonpolar metabolites that were released into the medium.

The conversion of 12-HETE to metabolic products has been described by several investigators. Using thin-layer chromatography (TLC), Stenson and Parker (4) found that human neutrophils release two polar metabolites into the extracellular medium. Pawlowski et al. (5) next reported 80% conversion of 12-HETE by human macrophages to one major and several minor polar metabolites. The high performance liquid chromatography (HPLC) retention time of the major metabolite and its ultraviolet absorbance maximum suggested that this compound was a dihydroxyeicosatetraenoic acid (diHETE), but no further characterization was performed. Birkle and Bazan (6) also observed that retinal tissue converts 12-HETE to

Abbreviations: 12-HETE, 12-S-hydroxy-5, 8, 10, 14-eicosatetraenoic acid; MDCK, Madin Darby Canine Kidney; HPLC, high performance liquid chromatography; GLC, gas–liquid chromatography; NDGA, nordihydroguaiaretic acid; TLC, thin-layer chromatography; diHETE, dihydroxyeicosatetraenoic acid; 15-HETE, 15-hydroxyeicosatetraenoic acid; 16:3 (8-OH), 8-hydroxyhexadecatrienoic acid; DPBS, Dulbecco's phosphate-buffered saline; BSA, bovine serum albumin.

[1]To whom reprint requests should be addressed at: Department of Internal Medicine, E 300 G GH, University of Iowa College of Medicine, Iowa City, IA 52242.

at least three polar radioactive metabolites. The most abundant metabolite had the chromatographic properties of a diHETE on normal phase HPLC (6). In addition, Marcus et al. (14, 15) and Wong et al. (16) have found that platelet-derived 12-HETE is converted to polar metabolites by human neutrophils. When 12-HETE was incubated with ionophore-stimulated neutrophils, activation of the neutrophil 5-lipoxygenase facilitated its conversion to 5, 12-diHETE (14). However, with unstimulated neutrophils, the major 12-HETE metabolite released into the medium was a cytochrome P-450 product 12(S) 20-diHETE produced through ω-oxidation (15, 16).

The above studies all indicate that the main product formed from 12-HETE is a diHETE. By contrast, Hadjiagapiou et al. (17) recently identified the major metabolite of 12-HETE produced by vascular smooth muscle cells as a monohydroxy fatty acid, 8-hydroxy-hexadecatrienoic acid [16:3 (8-OH)]. This compound, which has HPLC retention time similar to a diHETE, is thought to be produced by removal of 4 carbons from the carboxyl end of 12-HETE.

To determine which of these pathways is operative in epithelium, we identified the major metabolite of 12-HETE produced by renal epithelial cells. Like smooth muscle cells, the MDCK tubular epithelial cell line converts 12-HETE primarily to the monohydroxy fatty acid derivative, 16:3(8-OH). Furthermore, 16:3(8-OH) is released from both the apical and basolateral surfaces of the epithelium, suggesting that this product may have effects within the kidney parenchyma or renal microcirculation.

MATERIAL AND METHODS

Cell culture

MDCK cells were obtained from American Type Culture Collection (Rockville, MD) and stored in liquid nitrogen with 7% dimethyl sulfoxide (DMSO) and 20% fetal bovine serum. Cells were studied between passages 55 and 70 and grown according to methods previously described (18). Briefly, cells were grown to confluency in Eagle's minimal essential medium with Earle's salts and 20% heat-inactivated fetal bovine serum (Hy-Clone, Logan, UT) in tissue culture flasks with 75 cm^2 surface area (Corning Glass Works, Corning, NY), or in six-well tissue culture plates with a surface area of 10 cm^2/well (Tissue Culture Cluster 6, Costar, Cambridge, MA). Experiments were performed when the cultures were confluent and formed domes characteristic of MDCK cells (19). Cultures were placed in a temperature- and humidity-controlled incubator (CO_2 Incubator model 3028, Forma Scientific, Marietta, OH) at 37°C with 95% air–5% CO_2 as the gas phase.

Incubation and analysis

After removal of the maintenance medium, the cultures were washed twice with Dulbecco's phosphate-buffered saline (DPBS) solution with 0.1 μM bovine serum albumin (BSA). Cultures in the 75-cm^2 flasks were then incubated with 5.0 ml of 5.0 μM [^{3}H]12-HETE [12-(S)-HETE, 0.5 mg/ml, Cayman Chemical Corp.] and 0.5 μCi/ml 12 (S) hydroxy-[5, 6, 8, 9, 11, 12, 14, 15, (n)-^{3}H] eicosatetraenoic acid (119 Ci/mmol, Amersham Corp.) at 37°C for 1 h. After pooling the media from eight flasks (40 ml) into a large siliconized tube, the media was acidified to pH 3.5 with 12 N HCl, 10 ml ethyl acetate was added, and the tube was vortexed vigorously and centrifuged for 10 min at 400 *g* to separate the phases. The ethyl acetate was removed, and this extraction was repeated twice and the ethyl acetate extracts were pooled. The ethyl acetate was then evaporated under a stream of nitrogen, and the lipids were resuspended in 100 μl methanol–acetonitrile 50:50 (v/v).

High performance liquid chromatography (HPLC)

Lipids dissolved in methanol–acetonitrile were separated by reverse-phase HPLC with a Beckman 332 system equipped with a 4.5 × 150 mm column containing Adsorbosphere C_{18} reverse-phase 3-μm spherical packing (Alltech Associates, Deerfield, IL). The elution gradient contained water adjusted to pH 3.4 with phosphoric acid and acetonitrile (20). For isolation of the metabolites, the gradient was started with 27% acetonitrile and increased over 50 min to 100% acetonitrile. A linear gradient of 50–100% acetonitrile was used for further analysis of the metabolites. After the column effluent was mixed with Budget Solve scintillation fluid (RPI Corp., Mount Prospect, IL), the radioactivity was detected and assayed with a Radiomatic HPLC radioactivity flow detector (Radiomatic Flo One Beta, Radiomatic Instruments and Chemical Co., Tampa, FL).

Analytical HPLC was first carried out on a 2-μl aliquot to identify the retention time and calculate the amount of product formed. Fractions were collected from the remainder of the sample at 1-min intervals to isolate the peak corresponding to that of the major metabolite. This fraction, eluting at 25.0 min, was mixed with an equal volume of water and then isolated by extraction with 3 volumes of ethyl acetate.

Preparation of derivatives

After isolating the major metabolite by reverse-phase HPLC, it was methylated with diazomethane in ether (21). The methyl ester was converted to the trimethylsilyl ether by incubation with bis (trimethylsilyl) trifluroacetamide and 1% trimethylchlorosilane (Sylone BFT, Supelo, Inc., Bellefonte, PA) in pyridine (22). Catalytic hydrogenation was performed with 0.1 mg platinum oxide in 0.3 ml of

ethanol. After H_2 was bubbled through the solution for 1 min, the mixture was diluted with 0.7 ml of water and extracted 3 times with 1 ml ethyl acetate (23). The methyl esters dissolved in pyridine were acetylated by reaction with acetic anhydride for 2 h. After addition of cold water, the mixture was acidified to pH 3 with 2 N HCl and extracted 3 times with ether. The extracts were neutralized by washing before removal of the solvent (17). To check for the presence of keto groups, the purified metabolites dissolved in 50 μl of cold methanol were treated with 500 μg of sodium borohydride. After the mixture was allowed to warm to room temperature for 1 h, it was diluted with 500 μl of water, acidified to pH 3 with 2 N HCl, extracted with 1-butanol, neutralized, and dried by evaporation (17). To check for epoxides, the metabolites were treated with methanol–water 1:100 (v/v) (24).

Gas–liquid chromatography (GLC) and mass spectrometry

The hydrogenated and unhydrogenated methyl ester, trimethylsilyl ether derivatives of the main metabolite of 12-HETE were analyzed with a Riber R 10-10 quadropole mass spectrometer containing a 25 m × 0.2 mm GLC column packed with 5% phenylmethylsilicone maintained at 195°C. The energy of the electron beam was 22.5 eV. A spectrum of the hydrogenated sample was also obtained by chemical ionization with ammonia.

Incubation with inhibitors

Cultures grown in 6-well tissue culture plates were used for these experiments. The maintenance medium was removed and the cells were washed twice with DPBS containing 0.1 μM BSA. Nine hundred μl of modified Eagle's minimal essential medium containing 0.1 μM BSA supplemented with either 25 mM α-naphthoflavone, 20 μM nordihydroguaiaretic acid (NDGA), 0.1 mM 4-pentenoic acid (all purchased from Sigma Chemical Corp., St. Louis, MO), or 20 μl of ethanol as a control, was added to the cultures for 1 h. One hundred μl Eagle's minimal essential medium supplemented with [^{3}H]12-HETE (0.4 μCi/ml) was then added and the incubation was continued for 1 h. The medium was removed, acidified to pH 3.5 with 20 μl of 1 M citric acid, and the lipids were extracted with ethyl acetate as indicated above. The final extract was suspended in methanol–acetonitrile and an aliquot containing at least 15,000 dpm was separated by reverse-phase HPLC to detect the formation of metabolites.

Polarity of metabolite release

MDCK cells were grown on collagen-coated, fibronectin-treated polycarbonate filters (13). After confirming tightness of the epithelial junctions by measuring BSA transfer across the monolayer (25), the experiment was initiated by suspending the cylinder with the attached filter in a plastic tissue culture chamber containing DPBS; this solution was also added to the inside of the plastic cylinder. [^{3}H]12-HETE (0.5 μCi/ml) was then added to either the superior or inferior compartment. After 1 h incubation, the buffer solution from both compartments was removed, placed into individual siliconized vials, extracted with ethyl acetate, and assayed for radioactive metabolites by reverse-phase HPLC. The cylinder also was removed from the chamber and washed twice with buffer solution to remove any adherent medium. The filter then was removed, placed in a scintillation vial containing 4.0 ml of Budget Solve scintillation fluid, and assayed for radioactivity.

RESULTS

Formation of metabolites

Analysis of the incubation medium by reverse-phase HPLC revealed the progressive accumulation of several radiolabeled metabolites when MDCK cells were incubated with 1.0 μM [^{3}H]12-HETE for 1 h. This is illustrated in **Fig. 1**, which is a representative chromatogram. Of the total amount of radioactivity present in the medium, 19.4% remained as [^{3}H]12-HETE and 35.2% was recovered as the major product, designated as peak 2, which eluted at 24.8 min. In this HPLC system, 5, 12-diHETE (leukotriene B_4) eluted at 23.4 min and 12-

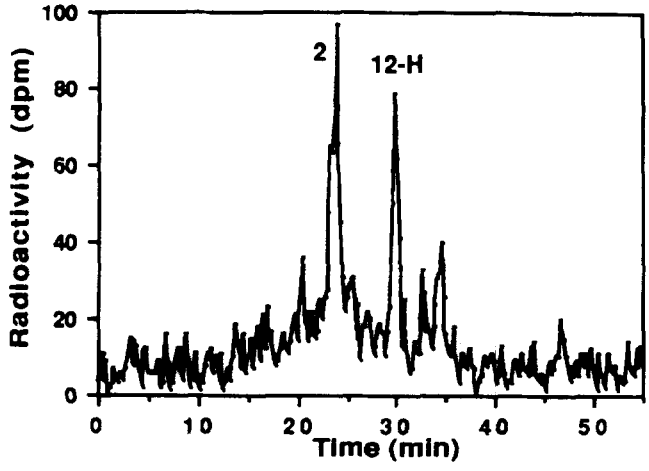

Fig. 1. HPLC profile of metabolic products formed by MDCK cells during 1 h incubation with 1.0 μM [^{3}H]12-HETE. MDCK cells were incubated with 1.0 [μM [^{3}H]12-HETE in serum-free modified Eagle's minimal essential medium with 0.1 μM BSA for 1 h. The medium was removed, centrifuged to remove any cells, acidified with 1 N citric acid, and the lipids were extracted with ethyl acetate. An aliquot was dried under N_2, resuspended in 27% acetonitrile in water, and the lipids were separated by reverse phase HPLC. Radioactivity was assayed with an on-line flow-through scintillation counter. 12-HETE (12-H) eluted at 30.6 min. Peak 2, the major metabolite produced by MDCK cells, eluted at 24.8 min.

hydroxy-5, 8, 10-heptadecatrienoic acid eluted at 26.4 min. This relative retention is similar to the elution profile previously reported by Hadjiagapiou et al. (17) for the major metabolite produced during incubation of [^{3}H]12-HETE with human umbilical smooth muscle cells.

Identification of the main metabolite

Following methylation and silylation of the material isolated from peak 2, GLC combined with mass spectrometry was used to identify this compound. **Fig. 2A** shows the electron impact mass spectrum containing a base peak at *m/z* = 241, consistent with an ion having the composition $+(CH_3)_3Si{=}OCH(CH{=}CH)_2(CH_2)_2\ COOH_3$. This suggests that 12-HETE was metabolized to an 8-hydroxyhexadecatrienoic acid [16:3(8-OH)], a conclusion supported by the presence of small ions at *m/z* = 305 and 337. These ions are formed by the loss of 47 and 15 mass units from a compound with a molecular weight of 352, the putative molecular weight of the derivatized metabolite.

The electron impact mass spectrum of the hydrogenated derivative of peak 2, shown in Fig. 2B, further confirms that the major metabolite is an 8-hydroxyhexadecatrienoic acid. This spectrum has major ions at *m/z* = 215 and 245. These ions were formed by cleavage on either side of the trimethylsilyl carbon atom. There is also an ion at 311, corresponding to a loss of 47 mass units from the parent compound. These ions, together with the major ions obtained with the native metabolite, indicate that the compound contains three double bonds. Two of these double bonds are present between the hydroxyl group and the carboxyl terminus, as indicated by the *m/z* = 245 and 241 ions obtained from the reduced and native compound, respectively.

The chemical ionization mass spectrum is shown in Fig. 2C. This was obtained with the hydrogenated, trimethylsilyl ether, methyl ester derivative of the compound using NH_3. The molecular ion at *m/z* = 359 corresponds to the protonated molecular weight of the methyl ester of the monohydroxy hexadecanoic acid.

Effects of metabolic inhibitors

The effect of various inhibitors on the conversion of [^{3}H]12-HETE to 16:3(8-OH) was tested. When no inhibi-

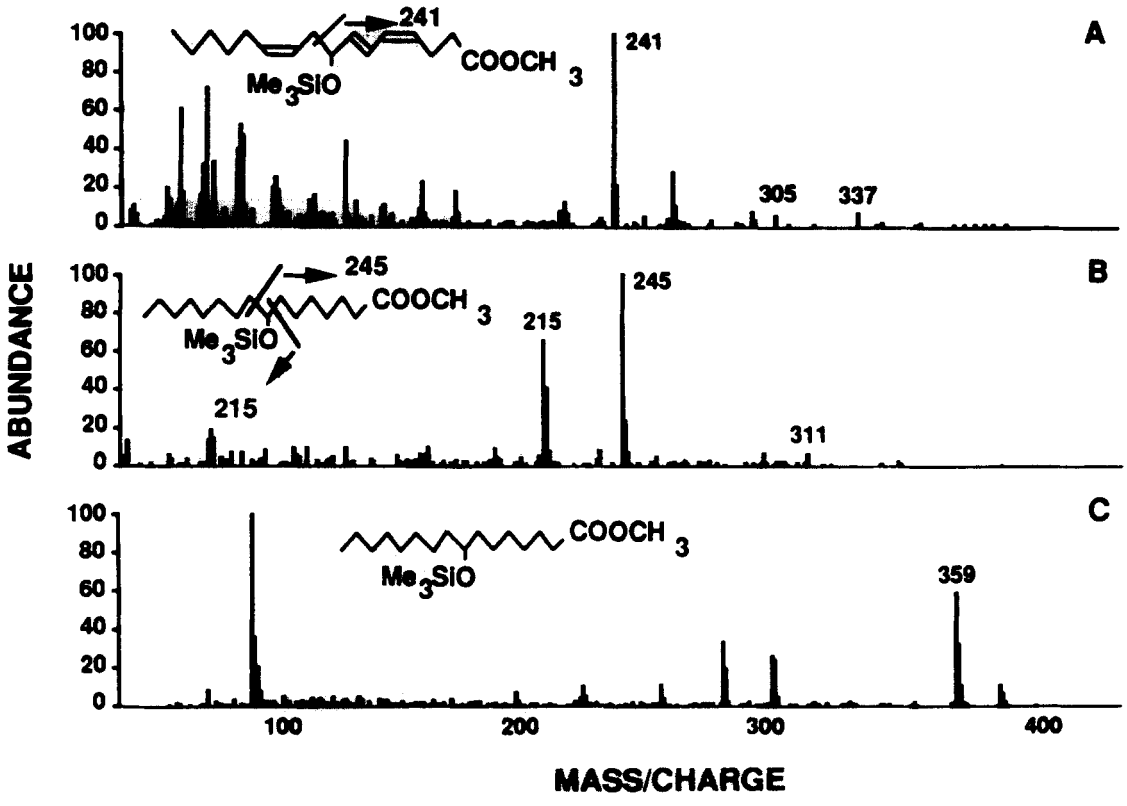

Fig. 2. Mass spectra of the trimethylsilyl ester, methyl ester derivative of the main metabolic product formed by MDCK cells from 12-HETE. This compound corresponds to peak 2 in Fig. 1, formed during incubation of MDCK cells with 12-HETE for 1 h. It was isolated by reverse phase HPLC, derivatized, and isolated by GLC. An electron impact mass spectrum of the trimethylsilyl ether, methyl ester derivative is shown in (A), an electron impact spectrum of the hydrogenated derivative in (B), and a chemical ionization spectrum of the hydrogenated derivative treated with NH_3 is shown in (C).

tors were present, MDCK cells converted 24.3% of the [^{3}H]12-HETE radioactivity to 16:3(8-OH). When incubated in the presence of 20 μM NDGA, a lipoxygenase and weak cyclooxygenase inhibitor, the conversion of [^{3}H]12-HETE was unimpaired, with 28.7% of the radiolabeled 12-HETE being converted to 16:3(8-OH). α-Naphthoflavone, an inhibitor of 12-HETE ω-oxidation (26), at a concentration of 25 mM also failed to inhibit the production of this metabolic product with greater than 30% of the radiolabeled 12-HETE being converted to 16:3 (8-OH). Likewise, 0.1 mM 4-pentenoic acid, an inhibitor of mitochondrial β-oxidation (27), failed to inhibit the production of 16:3(8-OH), with 20% of the native 12-HETE being converted to this compound. Furthermore, when 0.1 mM 4-pentenoic acid was incubated with MDCK cells for 2 h before addition of [^{3}H]12-HETE, no impairment in production of 16:3(8-OH) was observed. Also, no impairment in MDCK cell conversion to 16:3(8-OH) was observed when the 4-pentenoic acid concentration was raised to 0.2 or 0.5 mM. Taken together, these data suggest that 16.3(8-OH) is not synthesized by lipoxygenation, ω-oxidation, or mitochondrial β-oxidation.

Polarity of release of metabolite

Analysis of the media by reverse-phase HPLC revealed that release of 12-HETE metabolites from cells grown on micropore filters occurred in both the ipsilateral and contralateral directions. In these studies, MDCK cells grown on polycarbonate filters were incubated for 1 h with [^{3}H] 12-HETE added to either the apical or basal solutions. The contralateral release is illustrated in **Fig. 3**, which reveals a similar pattern of release of polar metabolites and small amounts of unmodified 12-HETE to both the bottom and top fluids when the [^{3}H]12-HETE was added to the opposite compartment. The major metabolite released in both directions, designated peak 2, had a retention time of 24.2 min, identical to the retention of peak 2 in Fig. 1 when the MDCK cells were grown on a plastic surface. Thus, the major metabolite of 12-HETE, 16:3(8-OH) is released into both the apical and basal solutions. **Fig. 4** compares the amount of 16:3 (8-OH) released to the apical and basal solutions following addition of [^{3}H]12-HETE to either the top or bottom compartment. When [^{3}]12-HETE was added to the top compartment, a significantly greater amount of 16:3(8-OH) was released to the basal as compared with the apical fluid (Fig. 4, left). Similarly, when [^{3}H]12-HETE was added to the bottom, there again was greater release of 16:3(8-OH) into the basal medium than the apical medium (Fig. 4, right). These findings suggest a preferential release of 16:3(8-OH) from the surface of the tubular epithelium that, in this model system, represents the direction of the kidney parenchyma and/or renal microcirculation.

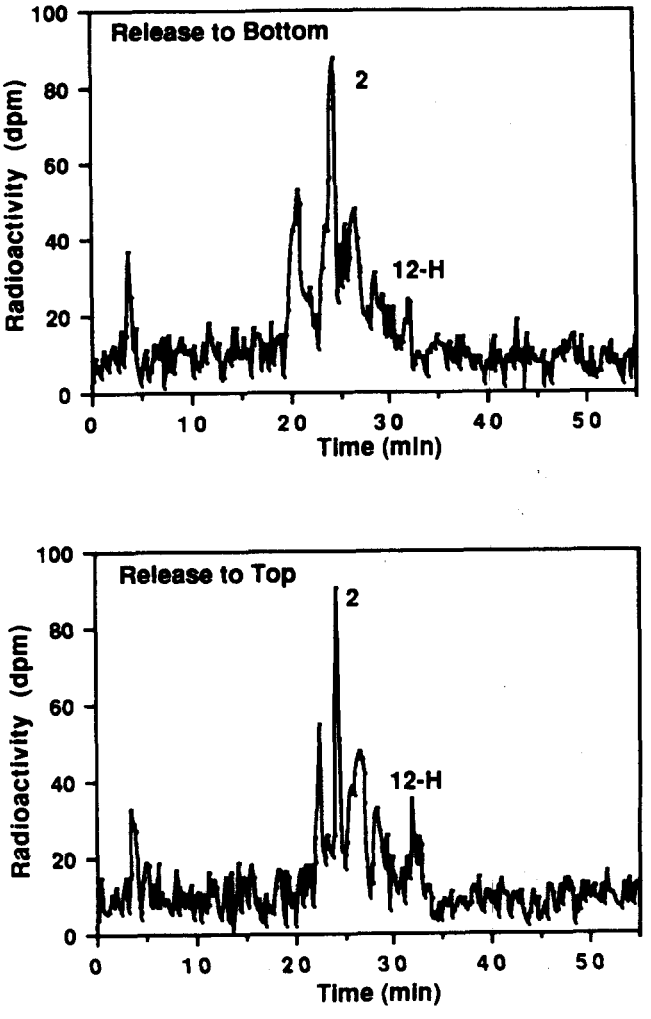

Fig. 3. HPLC profile of metabolic products released from MDCK cells grown on collagen-treated, fibronectin-coated polycarbonate filters. MDCK cells were grown on collagen-treated polycarbonate filters attached to 13-mm plastic cylinders. Cylinders were suspended in plastic tissue culture chambers, with a buffered salt solution present in compartments on both sides of the filter. In some chambers, [^{3}H]12-HETE was added to the top (apical) compartment; in others, to the bottom (basal) compartment. At the end of 1 h, the lipids were extracted from the contralateral medium, pooled, and an aliquot containing at least 5,000 dpm was injected into the reverse phase HPLC column. The metabolites released to the bottom compartment after addition of [^{3}H] 12-HETE to the apical fluid are shown on the top; the metabolites released to the top compartment after addition of [^{3}H]12-HETE to the basal fluid are shown on the bottom. 12-HETE (12-H) eluted at 32.4 min. Peak 2, the major metabolite of 12-H, eluted at 24.2 min.

DISCUSSION

These results demonstrate that the main metabolite formed from 12-HETE by cultured canine tubular epithelial cells, which comprises 35% of the radioactivity derived from [^{3}H]12-HETE during a 1 h incubation, is 16:3(8-OH). It most likely is 8-hydroxy-4,6,10-hexadecatrienoic acid (Fig. 2). Therefore, as opposed to conversion to a di-

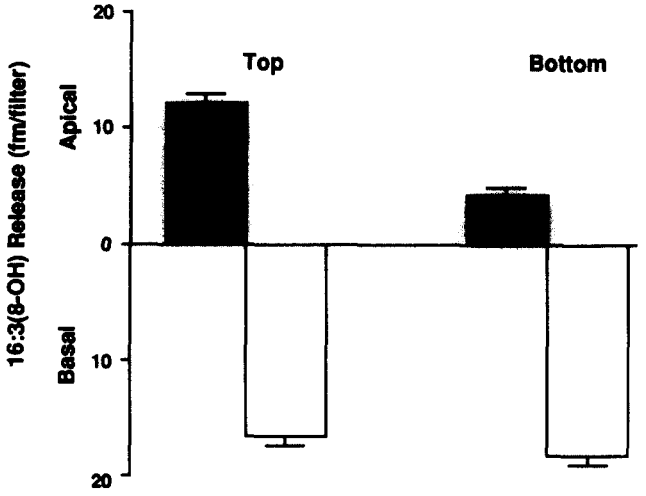

Fig. 4. Polarity of release of the major metabolite of 12-HETE produced by MDCK cells. MDCK cells were grown on collagen-treated polycarbonate filters and incubated with [^{3}H]12-HETE as described in Fig. 3. The [^{3}H]12-HETE was added to either the top compartment (left side) or the bottom compartment (right side). At the end of 1 h, the cylinder was removed, [^{3}H]12-HETE uptake on the filter was measured by counting the radioactivity on the filter, and the fluid above and below the filter was analyzed via HPLC. The amount of 16:3 (8-OH) released to either the apical or basal solution was quantitated by converting counts per minute (cpm) to femtamoles (fm) and standardized by dividing by the uptake on the filter after 1 h.

HETE as seen in neutrophils (14–16), 12-HETE is metabolized to a shorter-chain mono-HETE by this epithelial cell line. The metabolite apparently is formed by oxidative removal of 4 carbons from the carboxyl terminus of 12-HETE, as occurs in vascular smooth muscle cells (17). If this is the case, then the double bonds are likely to remain in the same positions as in 12-HETE, placing them in the 4-, 6-, and 10-positions of the newly formed 16-carbon product. This interpretation is entirely consistent with the mass spectral data, which confirmed the original conjugated diene structure and locates two of the double bonds between the carboxyl group and the hydroxylated carbon, and the third between the latter and the methyl terminus.

The mechanism responsible for conversion of 12-HETE to 16:3(8-OH) was not directly determined. However, neither an ω-oxidation nor a lipoxygenase inhibitor significantly reduced the conversion of [^{3}H]12-HETE to its major metabolite. Therefore, the most likely mechanism for the removal of the 4 carbons from the carboxyl end of 12-HETE to produce 16:3(OH) is a β-oxidation process.

β-Oxidation of the arachidonic acid lipoxygenase product, 15-hydroxyeicosatetraenoic acid (15-HETE), has been previously reported (28, 29). Shen et al. (28) found that 15-HETE was metabolized to an 11-hydroxy-hexadecatrienoic acid [16:3(11-OH)] when incubated with human umbilical vein endothelial cells. Salzmann-Reinhardt et al. (29) found that when [^{14}C]arachidonic acid was incubated with rabbit reticulocytes, 30% was first converted to 15-HETE, and 10-20% of this product was subsequently oxidized to $^{14}CO_2$. Hadjiagapiou et al. (17) also concluded that β-oxidation was responsible for 16:3(8-OH) formation in vascular smooth muscle. Therefore, β-oxidation appears to be a major route of metabolism of both 12- and 15-HETE in several different tissues, including endothelium, smooth muscle, and epithelium. If 12-HETE was oxidized to 16:3(8-OH) in the mitochondria, then 4-pentenoic acid, an inhibitor of mitochondrial β-oxidation, should have reduced this conversion. However, 0.1 mM 4-pentenoic acid appreciably reduced the production of 16:3(8-OH) by only 17%, and higher concentrations produced little additional effect.

The failure of 4-pentenoic acid to inhibit the conversion of 12-HETE to 16:3(8-OH) in the renal tubular epithelial cell differs from what has been reported for 15-HETE metabolism in human umbilical vein endothelial cells (28). Shen et al. (28) found that 0.05 mM 4-pentenoic acid decreased the production of 16:3(11-OH) from 15-HETE by 55%. The failure of 4-pentenoic acid to prevent 16:3(8-OH) production suggests that either the oxidative pathways for 12- and 15-HETE are different, or the pathway in endothelial cells differs from that in epithelial cells.

Recently, the role of peroxisomes in fatty acid β-oxidation has become partially clarified (30). In isolated rat liver, β-oxidation of long chain monounsaturated fatty acids appears to occur preferentially in peroxisomes, where the fatty acids are chain shortened, and then subsequently transported to the mitochondria where further β-oxidation takes place (31).

In view of the recent information that peroxisomes may also play a role in the metabolism of long-chain polyunsaturated fatty acids (32), we propose that 12-HETE is primarily β-oxidized in the peroxisomes of the tubular epithelial cells, rather than in the mitochondria. Apparently, the resulting 16:3(8-OH) is not a good substrate for further β-oxidation. If the subsequent pathway is similar to that reported for the monounsaturated fatty acids, one may speculate that 16:3(8-OH) either is not efficiently transported to the mitochondria or does not readily undergo mitochondrial β-oxidation.

Although the MDCK cells released 16:3(8-OH) to both the basal and apical compartments of the filter chamber (Figs. 3 and 4), they released considerably more to the basal fluid, regardless of whether 12-HETE was the top or bottom chamber (Fig. 4). If 16:3(8-OH) is simply an inert, metabolically inactive product of 12-HETE, one might anticipate that it would be released almost exclusively to the apical compartment, which in this model system represents excretion into the renal tubular fluid and, therefore, into the potential urinary space. Because more 16:3(8-OH) is released into the basal fluid, which in this model system represents the polarity of the kidney parenchyma

and circulation, one might speculate that this metabolite may have some heretofore unidentified functional significance. Further studies to investigate this question are currently in progress.

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REFERENCES

1. Needleman, P., J. Turk, B. A. Jakschik, A. R. Morrison, and J. B. Lefkowith. 1986. Arachidonic acid metabolism. *Annu. Rev. Biochem.* **55:** 69–102.
2. Hamberg, M., and B. Samuelsson. 1974. Prostaglandin endoperoxides. Novel transformation of arachidonic acid in human platelets. *Proc. Natl. Acad. Sci. USA.* **71:** 3400–3404.
3. Rabinovitch, H., J. Durand, M. Rigand, F. Mendy, and J-C. Bretow. 1981. Transformation of arachidonic acid into monohydroxy-eicosatetraenoic acids by mouse peritoneal macrophages. *Lipids.* **16:** 518–524.
4. Stenson, W. F., and C. W. Parker. 1979. 12-L-hydroxy-5, 8, 10, 14,-eixosatetraenoic acid is incorporated into neutrophil phospholipids and triglyceride. *Prostaglandins.* **18:** 285–292.
5. Pawlowski, N. A., W. A. Scott, M. Andreach, and A. Z. Cohn. 1982. Uptake and metabolism of monohydroxyeicosatetraenoic acids by macrophages. *J. Exp. Med.* **155:** 1653–1664.
6. Birkle, D. L., and N. G. Bazan. 1984. Effect of K^+ depolarization on the synthesis of prostaglandins and hydroxyeicosatetra (5, 8, 11, 14)enoic acids (HETE) in the rat retina. *Biochim. Biophys. Acta.* **795:** 564–573.
7. Metz, S. A. 1985. Glucose increase synthesis of lipoxygenase-mediated metabolites of arachidonic acid in intact rat islets. *Proc. Natl. Acad. Sci. USA.* **82:** 198–202.
8. Nadler, J. L., R. Natarajan, and N. Stern. 1987. Specific action of the lipoxygenase pathway in mediating angiotensin-II-induced aldosterone synthesis in isolated adrenal glomerulosa cells. *J. Clin. Invest.* **80:** 1763–1769.
9. Goetzl, E. J., J. M. Woods, and R. R. Gorman. 1977. Stimulation of human eosinophil and neutrophil polymorphonuclear leukocyte chemotaxis and random migration by 12-L-5, 8, 10, 14-eicosatetraenoic acid. *J. Clin. Invest.* **59:** 179–183.
10. Goetzl, E. S. 1980. A role for endogenous mono-hydroxy-eicosatetraenoic acids (HETES) in the regulation of human neutrophil migration. *Immunology.* **40:** 709–719.
11. Yamaja Setty, B. N., J. E. Graeber, and M. J. Stuart. 1987. The mitogenic effect of 15- and 12-hydroxyeicosatetraenoic acid on endothelial cells may be mediated via diacylglycerol kinase. *J. Biol Chem.* **262:** 17613–17622.
12. Hadjiagapiou, C., and A. A. Spector. 1986. 12-Hydroxyeicosatetraenoic acid reduces prostacyclin production by endothelial cells. *Prostaglandins.* **31:** 1135–1144.
13. Gordon, J. A. and A. A. Spector. 1987. Effects of 12-HETE on renal tubular epithelial cells. *Am. J. Physiol.* **253:** C277–C285.
14. Marcus A. J., M. J. Broekman, L. B. Safier, H. L. Ullman, N. Islam, C. N. Serhan, L. E. Rutherford, H. M. Korchak, and G. Weissman. 1982. Formation of leukotrienes and other hydroxy acids during platelet-neutrophil interactions in vivo. *Biochem. Biophys. Res. Commun.* **109:** 130–137.
15. Marcus, A. J., L. B. Safier, H. L. Ullman, M. J. Broekman, N. Islam, T. D. Oglesby, and R. R. Gorman. 1984. 12S, 20-Dihydroxyeicosatetraenoic acid: a new eixosanoid synthesized by neutrophils from 12-S-hydroxyeicosatetraenoic acid produced by thrombin on collagen-stimulated platelets. *Proc. Natl. Acad. Sci. USA.* **81:** 903–907.
16. Wong, P. Y-K., P. Westlund, M. Hamberg, E. Grandström, P. H-W. Chao, and B. Samuelsson. 1984. ω-Hydroxylation of 12-L-hydroxy-5, 8, 10, 14-eicosatetraenoic acid in human polymorphonuclear leukocytes. *J. Biol. Chem.* **259:** 2383–2386.
17. Hadjiagapiou, C., H. Sprecher, T. L. Kaduce, P. H. Figard, and A. A. Spector. 1987. Formation of 8-hydroxyhexadecatrienoic acid by vascular smooth muscle cells. *Prostaglandins.* **34:** 579–589.
18. Lewis, M., T. L. Kaduce, and A. A. Spector. 1981. Effect of essential polyunsaturated fatty acid modifications on prostaglandin production by MDCK canine kidney cells. *Prostaglandins.* **22:** 747–760.
19. Rabito, C. A., J. Tchao, J. Valentich, and J. Leighton. 1978. Distribution and characteristics of the occluding junctions in a monolayer of a cell line (MDCK) derived from canine kidney. *J. Membr. Biol.* **43:** 351–365.
20. Eling, T., B. Trainer, A. Ally, and R. Warnock. 1982. Separation of arachidonic metabolites by high-performance liquid chromatography. *Methods Enzymol.* **86:** 511–517.
21. Fales, H. M., T. M. Jaouni, and J. F. Babaskak. 1973. Simple device for preparing ethereal diazomethane without resorting to codistillation. *Anal. Chem.* **45:** 2302–2303.
22. Maas, R., L. J. Turk, J. A. Oates, and A. R. Brash. 1982. Formation of a novel acid from arachidonic acid by lipoxygenase-catalyzed double oxygenation in rat mononuclear cells and human leukocytes. *J. Biol. Chem.* **257:** 7056–7062.
23. Granstrom, E., and B. Samuelsson. 1971. On the metabolism of prostaglandin $F_{2\alpha}$ in female subjects. II. Structures of six metabolites. *J. Biol. Chem.* **246:** 7470–7485.
24. Hamberg, M., C. A. Herman, and R. P. Herman. 1986. Novel biological transformations of 15-L_s -hydroperoxy-5, 8, 11, 13-eicosatetraenoic acid. *Biochim. Biophys. Acta.* **877:** 447–457.
25. Henning, B., D. M. Shasby, A. B. Fulton, and A. A. Spector. 1984. Exposure to free fatty acid increases the transfer of albumin across cultured endothelial monolayers. *Arteriosclerosis.* **4:** 489–497.
26. Marcus, A. J., L. B. Safier, H. L. Ullman, N. Islam, M. J. Broekman, and C. von Schacky. 1987. Studies on the mechanism of ω-hydroxylation of platelet 12-hydroxyeicosatetraenoic acid (12-HETE) by unstimulated neutrophils. *J. Clin. Invest.* **79:** 179–187.
27. Schutz, H. 1987. Inhibitors of fatty acid oxidation. *Life Sci.* **40:** 1443–1449.
28. Shen, X-Y., P. H. Figard, T. L. Kaduce, and A. A. Spector. 1987. Conversion of 15-hydroxyeicosatetraeonic acid to 11-hydroxyhexadecatrienoic acid by endothelial cells. *Biochemistry.* **27:** 996–1004.
29. Salzmann-Reinhardt, U., H. Kühn, R. Wiesner, and S. Rapoport. 1985. Metabolism of polyunsaturated fatty acids by rabbit reticulocytes. *Eur. J. Biochem.* **153:** 189–194.

30. Alexon, S. E. H., and B. Cannon. 1984. A direct comparison between peroxisomal and mitochondrial preferences for fatty-acyl beta-oxidation predicts channelling of medium-chain and very long-chain unsaturated fatty acids to peroxisomes. *Biochim. Biophys. Acta.* **796:** 1–10.
31. Tolbert, E. 1981. Metabolic pathways in peroxisomes and glyoxysomes. *Annu. Rev. Biochem.* **50:** 133–157.
32. Hiltunen, J., K. T. Karki, I. E. Hassinen, and H. Osmundsen. 1987. Oxidation of polyunsaturated fatty acids by rat liver peroxisomes. *J. Biol. Chem.* **261:** 16484–16493.