Biosynthesis of 18(RD)-Hydroxyeicosatetraenoic Acid from Arachidonic Acid by Microsomes of Monkey Seminal Vesicles

SOME PROPERTIES OF A NOVEL FATTY ACID \( \omega_3 \)-HYDROXYLASE AND \( \omega_3 \)-EPOXYGENASE

(Received for publication, April 11, 1989)

Ernst H. Oliw
From the Department of Pharmacology, Karolinska Institutet, Box 60460, 104 01 Stockholm, Sweden

Microsomes of seminal vesicles of the cynomolgus monkey were incubated with \(^{14}C\)5,8,11,14-eicosatetraenoic (arachidonic) acid and NADPH for 40 min at 37 °C and the products were characterized. Prostaglandins \( F_2 \alpha \) and \( E_2 \) were the two main metabolites (\( \sim 52\% \) of radioactivity), while 18(R)-hydroxy-cis-5,8,11,14-eicosatetraenoic acid (18(R)-HETE) was identified as the main, less polar product (\( \sim 13\% \)). Significant biosynthesis of the 19-hydroxy or 20-hydroxy metabolites of arachidonic acid could not be detected. The formation of 18(R)-HETE was further investigated in the presence of a prostaglandin synthesis inhibitor, diclofenac sodium. The \( \omega_3 \)-hydroxylation was only partly supported by substituting NADH for NADPH. The hydroxyl oxygen of 18(R)-HETE was derived from the atmosphere and the \( \omega_3 \)-hydroxylation was inhibited by proadifen and partly inhibited by carbon monoxide. These findings suggest that 18(R)-HETE is formed by a cytochrome P-450 (P-450\(_{18,3}\)). Linoleic acid and 5,8,11,14-eicosatetraenoic acid were also substrates of the enzyme, but stearic acid was not metabolized. 5,8,11,14,17-Eicosatetraenoic acid was oxygenated under these conditions mainly to 17,18-dihydroxy-5,8,11,14-eicosatetraenoic acid, presumably formed from 17(18)-epoxy-5,8,11,14-eicosatetraenoic acid by hydrolysis. The seminal microsomes thus seem to possess both \( \omega_3 \)-hydroxyrase and \( \omega_3 \)-epoxygenase activity. These seminal vesicles also contain prostaglandin E 19-hydroxylase (Oliw, E. H., Kinn, A.-C., and Kvist, U. (1988) J. Biol. Chem. 263, 7222-7227). The presence of arachidonate \( \omega_3 \)-hydroxylase and prostaglandin E 19-hydroxylase was assessed in microsomes of adult and juvenile monkey livers. Arachidonic acid was metabolized extensively to diols (via epoxides), but 18-HETE could not be detected. In contrast, prostaglandin E\(_1\) was slowly hydroxylated mainly to 19-hydroxyprostaglandin E\(_1\) by both adult male and female juvenile hepatic microsomes. The results indicate that P-450\(_{18,3}\) of seminal vesicles might be a tissue-specific enzyme.
invalens in the presence of NADPH. Some properties of the microsomal enzyme, which seems to be a cytochrome P-450 isozyme, designated P-450\(_{\text{ONO}}\), is described. The enzyme seems to be relatively insensitive to the presence of inorganic anions and appears to differ in this respect from a fungal enzyme with both \(\omega-2\) and \(\omega-3\) hydroxylase activity, which was described by Sih et al. (23).

A secondary goal was to estimate to what extent the two seminal enzymes, P-450\(_{\text{ONO}}\) and P-450\(_{\text{PP}}\), or related enzymes, are present in primate liver. Hydroxylation of various prostaglandins in the liver has been studied extensively in rodents and other species, which lack seminal prostaglandin hydroxylases (6), but not in primates or sheep, which possess these seminal enzymes. Many male genital enzymes, including PGE-19-hydroxylase of seminal vesicles, are induced by testosteregione (24). Testosterone may also induce expression of these enzymes in other tissues. It therefore seemed of interest to compare adult male and juvenile female primate liver with regard to the metabolism of PGE, and arachidonic acid.

**MATERIALS AND METHODS**

**Materials**—[\(1^\text{14C}\)]Arachidonic acid (55–58 Ci/mol) was purchased from Amersham Corp. Arachidonic acid was purchased from Sigma and purified by silicic acid chromatography. [\(2^\text{14C}\)]PGE (n-6), [\(1^\text{14C}\)]PGB (n-6), and [\(1^\text{14C}\)]PGE (n-3) were gifts of Drs. J. E. Pike and J. C. Sih of the Upjohn Co. 20-Hydroxy-PGE\(_2\) was a gift of Dr. M. Toubouche, of the ONO Pharmaceutical Co., Osaka, Japan. 19(R)-Hydroxy-PGE\(_2\) and [\(2^\text{14C}\)]20:3 (n-6), [\(1^\text{14C}\)]20:4 (n-6) were gifts of Dr. J. F. Smith, French National Institute for Agricultural Research, Paris. 18:2 (n-6) and 18:3 (n-3) were gifts of Dr. J. F. Kline, Welwyn, England. 18:2 (n-6) was from Sigma, and 18:3 (n-3) was from Croda Chemicals, Limburg, Netherlands. All other chemicals and all solvents for HPLC (LiChrosolv) were from Merck, Darmstadt, FRG.

**Experimental—Tissues of cynomolgus monkeys, Macaca fascicularis**, were purchased from Statens Bakteriologiska Laboratorium, Solna, Sweden. The animals were killed by injection of pentobarbital sodium. The seminal vesicles (usually weighing 6–10 g) and the livers were obtained at 37 °C, centrifuged at 10,000 X g, 20 min; 100,000 X g, 60 min; + 4 °C) of a 10% homogenate of the tissue was assayed. The enzymatic reaction was stopped by addition of ethanol (0.5%). A mass spectrum of each metabolite was obtained by GC-MS analysis with electron impact ionization (70 eV) was used for analysis of mono- and dihydroxy fatty acids.

**Analyses**—Equipment for HPLC consisted of injectors (Rheodyne 7125), pumps (Constamatic III and CM 4000, LDC/Milton Roy), and a UV detector (Kratos Spectroflow 757, set at 254 nm). All separations were performed with a capillary GC column (approximately 25-m SPB-1, 4.6 mm, 5-μm Nucleosil 50-5, eluted with 0.5% isopropyl alcohol in dichloromethane). Hydroxy fatty acids were analyzed by gas chromatography-mass spectrometry (GC-MS). Hydroperoxide standards were prepared as described (26), dissolved in CHCl\(_3\) (0.3 ml), and were suspended in buffer (0.05 M Tris-HCl (pH 7.4), containing KC\(_2\) (150 mM), EDTA (1 mM), EGTA (1 mM), dithiothreitol (0.5 mM), and phenylmethylsulfonyl fluoride (0.1 mM)). In some experiments with seminal microsomes, diclofenac sodium was dissolved in water and added to a final concentration of 0.3 mM in order to inhibit prostaglandin biosynthesis. For product identification, 3-4 ml of the microsomal suspension (1,1–1.3 mg protein/ml) was incubated with 0.1 mM 20:4 (n-6) or 0.1 mM 20:5 (n-3), while 1–2 ml of the same suspension was incubated with 1–1.5 μCi of [\(2^\text{14C}\)]20:4 (n-6) or [\(1^\text{14C}\)]20:5 (n-3). The fatty acids were added in ethanol (<0.5%). After 40 min at 37 °C, the radioactive and unlabeled incubations were combined and terminated with ethanol. The effect of CO was assessed by mixing 4 volumes of ice-cold buffer, saturated with either CO or nitrogen, with 1 volume of suspended microsomes in ice cold aerated buffer (13). The final concentrations of [\(1^\text{14C}\)]20:4 (n-6) or [\(2^\text{14C}\)]20:5 (n-3) and NADPH were 2.5 μmol and 1 μmol, respectively, and the samples were then incubated at 37 °C for 30 min (14). 17(S)-Hydroxy-4,7,10,13,15,19-docosahexaenoic acid (0.5–1 μmol) was added in the internal standard, and the recovery of the internal standard was assessed by UV analysis at 237 nm during RP-HPLC. Effects of inhibitors and cofactors on the metabolism of [\(1^\text{14C}\)]20:4 or [\(2^\text{14C}\)]20:5 (6–12 μmol) were assessed in duplicate with the use of the internal standard.
applied to a cartridge of silicic acid (SepPak, Waters Associates), which was eluted with 4.5 ml of CHCL. These derivatives were separated on a capillary GC column (30-m DB5, 0.25-μm film thickness, J&W Scientific, with helium as carrier; the GC was programmed from 60 to 275 °C with 25 °C/min) and analyzed on an Incon 50 quadrupole mass spectrometer (Finnigan) as above. C values were estimated from the retention times on GC of saturated fatty acid methyl esters. Radioactivity was measured by liquid scintillation (Packard Tri-Carb 3375) using Ready-Solv HP (Beckman) as scintillation fluid. UV spectra were recorded with a Shimadzu 210A spectrophotometer and P-450 of liver microsomes was estimated as described (29). Protein was estimated by the Bradford assay (30).

RESULTS

Metabolism of Arachidonic Acid by Microsomes of Monkey Seminal Vesicles and NADPH

[14C]Arachidonic acid was metabolized by microsomes of seminal vesicles and NADPH to polar metabolites as shown in Fig. 1A. PGF₂α, and PGE₂ were identified in the main peak, which eluted between 10-22 ml and contained about 52% of recovered radioactivity. The peak of less polar material (70-80 ml, about 13% of the radioactivity) was found by SP-HPLC (Fig. 1B) and by GC-MS analysis to contain 18(R)-HETE as the main product. Unchanged [14C]arachidonic acid (about 35% of radioactivity) was eluted from the column with methanol. Finally, a few percent of radioactivity eluted with the same elution volume on RP-HPLC as 14,15-dihydroxy-5,8,11-eicosatrienoic acid (46 ml), but this material was not further characterized.

The biosynthesis of prostaglandins was inhibited by 0.3 mM diclofenac sodium, while the formation of 18(R)-HETE appeared to be unaffected. The biosynthesis of 18(R)-HETE was further studied with this inhibitor present and with the use of 17(S)-hydroxy-4,7,10,13,15,19-docosahexaenoic acid as an internal standard. In the presence of NADPH, the hydroxylation proceeded fairly linearly for 20-30 min and the rate then declined. 6, 12, and 36 μM [14C]arachidonic acid was found to be metabolized to [14C]18(R)-HETE at a mean rate of 10, 18, and 55 pmol/min/mg protein during 20 min of incubation. Without NADPH, the formation of 18(R)-HETE was insignificant, and NADH only partly supported the hydroxylation (Table I). The biosynthesis appeared to be partly reduced by carbon monoxide, while proadifen was a more effective inhibitor (Table I). PGE₅ (0.2 mM) also appeared to reduce the biosynthesis of 18(R)-HETE (by about 40%).

GC-MS Analysis of 18(R)-HETE

A mass spectrum of the Me₅Si ether methyl ester derivative is shown in Fig. 2A. The C value was 22.0 and, after hydrogenation, the C value was 22.6. A mass spectrum of the same derivative of the hydrogenated compound is shown in Fig. 2B. Both mass spectra showed a prominent signal at m/z 131 (CH(O'Si(CH₃)₃)-CH₂-CH₃). The unsaturated mass spectrum showed signals, among others, at m/z 406 (M'), 391 (M' -15), 377 (M' -29, loss of CH₃-CH₂), 375 (M' -31, a weak signal), 349 (M' -48, unidentified, possibly due to loss of OCH-CH₂-CH₃ with migration of Me₅Si to the carboxyl), 316 (M' -90), 287 (377-90), 285 (375-90), and in the lower mass range at m/z 145, 131 (base peak), 119, 105, 91, and 73. The saturated compound showed weak signals at m/z 399 (M' -15) and m/z 383 (M' -31), while strong signals were noted, among others, at m/z 385 (M' -29, loss of CH₃-CH₂), 356 (M' -58), 131 (CHO'Si(CH₃)₃)-CH₂-CH₃), and 73.

Mass spectra of the d₅-Me₅Si ether methyl ester derivative of the compound before and after hydrogenation are shown in the Miniprint and support the structure. The mass spectra of the unsaturated compound showed important signals at m/z 415 (M'), 397 (M' -18, loss of CH₃), 386 (M' -29), 357 (M' -58), 316 (M' -99, loss of HO(CH₂)₃(CH₃)₃), 287 (386 -99), 285 (M' -31+99)), and in the lower mass range at m/z 140 (CH(O'Si(CH₃)₃)-CH₂-CH₃), 119, 105, and 82 (base peak). The mass spectrum of same derivative of the saturated compound showed signals at m/z 405 (M' -18), 394 (M' -29), 377 (M' -31), 356 (M' -48), 349 (M' -63, a weak signal), 322 (M' -81), 316 (M' -99), and in the lower mass range at m/z 145 (CH(O'Si(CH₃)₃)-CH₂-CH₃), 119, 105, and 82.

Table I

<table>
<thead>
<tr>
<th>18(R)-HETE</th>
<th>17,18-dihydroxyeicosatetraenoic acid by microsomes of seminal vesicles: effects of cytochrome P-450 inhibitors and NADH</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADPH (1 mM)</td>
<td>100 ± 7 8 100 ± 5 6</td>
</tr>
<tr>
<td>Proudifen (1 mM)</td>
<td>24 ± 8 6 18 ± 6 6</td>
</tr>
<tr>
<td>Proudifen (0.3 mM)</td>
<td>74 ± 14 4</td>
</tr>
<tr>
<td>NADH (1 mM)</td>
<td>20 ± 1 6 31 ± 9 6</td>
</tr>
<tr>
<td>Buffer with O₂/N₂</td>
<td>100 ± 12 6 100 ± 7 3</td>
</tr>
<tr>
<td>Buffer with CO/O₂/N₂</td>
<td>63 ± 4 6 66 ± 16 4</td>
</tr>
</tbody>
</table>

An equal mixture of [14C]20:4 and [14C]20:5 as substrates

18(R)-Hydroxyeicosatetraenoic Acid

17847
The mass spectrum of the metabolite was found to be identical with that of the seminal metabolite. The configuration of the hydroxyl at C-18 was determined by capillary GC-MS of diastereoisomeric 2(S)-phenylpropiolic acid (PP) derivative of 18-hydroxyeicosanoic acid methyl ester (26). The latter was prepared by hydrogenation of 18-HETE methyl ester. The PP derivative of the racemic standard (methyl-[18-2H]18-hydroxyeicosanoate) was separated by capillary GC as shown in Fig. 4A. The major part (>90%) of the PP derivative of hydrogenated 18-hydroxyeicosatetraenoic acid of seminal origin had the same retention time on capillary GC as the second eluting isomer of the racemic standard (Fig. 3, B and C), i.e. the PP derivative of 18(RD)-hydroxyeicosanoate, derived by hydrogenation of 18-HETE, which was isolated from incubation with seminal microsomes. 

PGF_{2α} and PGE_{2}

These two compounds were identified in the polar fractions by mass spectra of the Me3Si ether methyl ester derivatives.

1 The optical purity of the biological products may be slightly higher than indicated by the selective ion chromatograms, since 2(S)-phenylpropionic acid (Aldrich, 97%) may contain a few percent of the 2(R)-isomer as judged from the steric analysis of optically pure standards (17(RD)-hydroxyeicosanoic acid methyl ester, 2(SO)-tetracosanol, and 2(RD)-tetracosanol (26, 37) kindly provided by Dr. M. Hamberg, Karolinska institutet.4
(31). PGE$_{15}$ was the main product as could be expected from the experimental conditions.

**Metabolism of Other Fatty Acids by Microsomes of Seminal Vesicles**

The metabolism of $[^4$C]$8,11,14$-eicosatrienoic acid and $[^4$C]$linoleic$ acid was assessed by incubating the fatty acids with seminal microsomes, 1 mM NADPH, 0.3 mM diclofenac sodium, and analyzing the products by RP-HPLC. In both cases polar metabolites were formed in high yields, and they had the same elution volumes on RP-HPLC as a mixture of the radiolabeled $\omega2$- and $\omega3$-hydroxy fatty acids, which were obtained by fungal biosynthesis from the radiolabeled fatty acids. Without addition of NADPH, virtually no products were formed. The structure of the seminal linoleic acid metabolites was confirmed by GC-MS to be 16-hydroxy-9,12-octadecadienoic acid (82%), but a significant amount of 17-hydroxy-9,12-octadecadienoic acid was also detected (about 18%). Linoleic acid was thus hydroxylated with less position specificity than arachidonic acid.

$[^4$C]Stearic acid was not metabolized to any significant degree by seminal microsomes and NADPH. Less than 1% of recovered radioactivity eluted with the same retention time on RP-HPLC as did a mixture of radiolabeled 16-hydroxy- and 17-hydroxy-stearic acids and biosynthesis of any other polar metabolites was not noted.

$[^4$C]$5,8,11,14,17$-Eicosapentaenoic acid was metabolized under the above conditions to a major product, which was much more polar than 14,15-dihydroxyeicosatrienoic acid on RP-HPLC. The elution volume of the metabolite was 37 and 7 ml for a semipreparative and an analytical column, respectively. The biosynthesis of this metabolite occurred only in the presence of NADPH. The product was identified as 17,18-dihydroxy-cis-5,8,11,14-eicosatetraenoic acid by GC-MS. Co-factor requirement and effects of cytochrome P-450 inhibitors on the biosynthesis of the metabolite are summarized in Table I. Incubation of an equal mixture of $[^4$C]icosapentaenoic acid and $[^4$C]arachidonic acid yielded 18(R)-HETE as the major product.

**GC-MS Analysis of 17,18-Dihydroxy-5,8,11,14-eicosatetraenoic acid**

The Me$_3$Si ether methyl ester derivative (Fig. 5A) showed signals at m/z 494 (M$^+$), 463 (M$^+$ -31), 404 (M$^+$ -90), 363 (M$^+$ -131, cleavage between C17 and C18), 334 (re-arrangement, loss of C17-C20 with transfer of Me$_3$Si to the carboxyl (32)), 233 (cleavage between C16 and C17), 143 (233 - 90), 131 (CH$_2$(O$^-$Si(CH$_3$)$_3$)-CH$_2$-CH$_3$) and 73. The d$_9$-Me$_3$Si derivative (Fig. 5B) showed signals at m/z 512 (M$^+$), 413 (M$^+$ -99), 372 (363 + 9), 343 (334 + 9), 315, 273 (372 - 99), 251 (233 + 18), 140 (131 + 9), and 82. After hydrogenation, strong signals were noted at m/z 471 (M$^+$ -31), 371 (M$^+$ -131), 149, 131 (CH$_2$(O$^-$Si(CH$_3$)$_3$)-CH$_2$-CH$_3$), and 73. The d$_9$-Me$_3$Si derivative showed, among others, information signals at m/z 480 (471 + 9) and 140 (131 + 9). From these data, from published mass spectra of other dihydroxy fatty acids with hydroxyls at the $\omega3$ and $\omega4$ carbons (31, 32), and assuming the position of the double bonds to be unchanged, the metabolite was identified as 17,18-dihydroxy-cis-5,8,11,14-eicosatetraenoic acid.

17,18-Dihydroxyeicosatetraenoic acid was also formed as the major product when eicosapentaenoic acid was incubated with *G. graminis*. The fungal and the seminal metabolite showed identical mass spectra.

**Metabolism of Arachidonic Acid, PGE$_5$, and PGB$_1$ by Monkey Liver Microsomes**

$[^4$C]Arachidonic acid was incubated with microsomes of adult male liver and NADPH. The fatty acid was metabolized extensively to polar products in all experiments. The products were separated by RP-HPLC, as shown in Fig. 6A, into fractions containing triols (about 10%), fractions containing the four vicinal diols (together about 50%) and fractions containing other metabolites (about 10%). The material in the major peaks of radioactivity was determined by GC-MS. PGE$_1$ was metabolized in low yields by adult male liver microsomes into 19-hydroxy-PGE$_1$ as the main product but 20-hydroxy-PGE$_1$ and 18-hydroxy-PGE$_1$ were also formed. After alkali treatment, the corresponding PGB compounds were partly separated by RP-HPLC as shown in Fig. 6B. The relative amounts of 20-hydroxy-PGB$_1$ and 19-hydroxy-PGB$_1$ were estimated by GC-MS. RP-HPLC and GC yielded approximately 10% 20-hydroxy-PGB$_1$, 75% 19-hydroxy-PGB$_1$, and 15% 18-hydroxy-PGB$_1$. The rate of formation of 19-hydroxy-PGB$_1$ was estimated to be 0.67 nmol/nmol cytochrome P-450 in 20 min. In contrast, PGB$_1$ was efficiently metabolized to 20-hydroxy-PGB$_1$ as the main product, followed by 19-hydroxy-PGB$_1$ and only trace amounts of 18-hydroxy-PGB$_1$ (Fig. 6C).

Liver microsomes of juvenile female monkeys metabolized $[^4$C]arachidonic acid and PGB$_1$ into essentially the same pattern of metabolites as shown for adult male liver. However,
judged from occurrence of certain fragments at the correct C
tives of the triol fractions (Fig. 6A) were analyzed by GC-MS. The most
polar peak of the two peaks contained 11,12,20-
the results were in agreement with published data (7, 10).
enoic acid was identified in the less polar peak (mass
spectrum on RP-HPLC as
which were identified by their C values and mass spectra of
amounts of
and C value as previously reported (10)).
of arachidonic acid (cf. Fig. 6A) were identified by com-
slowly (in the order of 0.2 nmol of 19-hydroxy-PGE\_1/nmol of
P-450/20 min).

**GC-MS Analysis of Hepatic Arachidonic Acid Metabolites**

**Triols**—Only the two major peaks of radioactivity, which were
resolved on rechromatography of methyl ester derivatives of the triol fractions (Fig. 6A) were analyzed by GC-MS. The most polar peak of the two peaks contained 11,12,20-trihydroxy-5,8,11-eicosatrienoic acid and much smaller amounts of 11,12,19-trihydroxy-5,8,11-eicosatrienoic acid, which were identified by their C values and mass spectra of Me\_3Si ether methyl ester derivatives in agreement with published data (8). Similarly, 5,6,20-trihydroxy-8,11,14-eicosatrienoic acid was identified in the less polar peak (mass spectrum and C value as previously reported (10)).

**Vicinal Diols and Other Products**—The four vicinal diols of arachidonic acid (cf. Fig. 6A) were identified by complete mass spectra (Me\_3Si ether methyl ester derivatives) and by their retention times on capillary GC (C values) as 14,15-dihydroxy-5,8,11-eicosatrienoic acid, 11,12-dihydroxy-5,11,14-eicosatrienoic acid, 8,9-dihydroxy-5,11,14-eicosatrienoic acid, and 5,6-dihydroxy-8,11,14-eicosatrienoic acid, and the results were in agreement with published data (7, 10).

5,6-Dihydroxy-8,11,14-eicosatrienoic acid had a similar elution volume on RP-HPLC as 18-hydroxy-, 19-hydroxy-, and 20-hydroxyeicosatetraenoic acids, but the two first compounds could not be detected in hepatic fractions. The Me\_3Si ether methyl ester derivative of 20-hydroxyeicosatetraenoic acid gives rise to a rather noncharacteristic mass spectrum (7), but with a characteristically large C value (22.6). Small amounts of this compound may have been present, as judged from occurrence of certain fragments at the correct C value during GC-MS analysis. However, analysis of the triols above showed that \(\omega\)- and \(\omega\)-hydroxylase activities were present in the hepatic microsomes.

**DISCUSSION**

The new finding of the present study is the existence of a regiospecific and prominent arachidonic acid \(\omega\)-hydroxylase in a mammalian tissue, the seminal vesicles of the cynomolgus monkey (M. fascicularis). The fatty acid \(\omega\)-hydroxylase incorporates atmospheric oxygen into the substrate and requires NADPH for full catalytic activity. Furthermore, the enzyme is inhibited by proprafen (SKF 5252A) and, to some extent, by carbon monoxide. The \(\omega\)-hydroxylase is therefore likely a cytochrome P-450 isozyme, designated \(P-450_{\omega}\), with an apparently unique regiospecificity, since biosynthesis of \(\omega\)- and \(\omega\)-hydroxylations of arachidonic acid was insignificant.

Polyunsaturated fatty acids of the (n-6) series appeared to be substrates of P-450\(_{\omega}\), although linoleic acid was metabolized with less position specificity. A saturated fatty acid, stearic acid, was not metabolized, while a fatty acid of the (n-3) series, eicosapentaenoic acid, was transformed by the microsomes by \(\omega\)-epoxidation rather than \(\omega\)-hydroxylation. Whether \(\omega\)-hydroxylation of arachidonic acid and the \(\omega\)-epoxidation of eicosapentaenoic acid are catalyzed by one enzyme or by two different enzymes of seminal vesicles will await a more detailed analysis (cf. Table I). The reactions catalyzed by the enzyme(s) are summarized in Fig. 7.

Seminal fluid of man and primates contain 19-hydroxy-PGE\_1 and 19-hydroxy-PGE\_2 in large amounts. They are formed by 19-hydroxylation of prostaglandins in the seminal vesicles by P-450\(_{18}\). An intriguing question is whether the seminal P-450\(_{18}\) and P-450\(_{18}\) could be the very same enzyme. In view of the strict substrate specificity of P-450\(_{18}\), its rather low enzyme activity and its primary oxygenation at the \(\omega\) carbon, this seems unlikely. Seminal vesicles also possess PGE 18-hydroxylase. A few percent of total hydroxy PGE compounds in human seminal fluid is actually 18-hydroxy-PGE\_1 and 18-hydroxy-PGE\_2 (17). It seems more likely that fatty acid \(\omega\)-hydroxylase and PGE 18-hydroxylase might be related. It should be added in this context that the catalytic activity of P-450\(_{18}\) and of P-450\(_{18}\) are 1-2 orders of magnitude higher than that of the PGE 18-hydroxylase.

Epoxidation of polyunsaturated fatty acids occurs in rodent liver (7-9) and a cytochrome P-450-dependent arachidonic acid epoxygenase was recently described in human liver (33). Epoxidation of arachidonic acid was found in this report to be a dominating pathway in microsomes of monkey liver. The monkeys were caught wild and little is known about their drug history in captivity. It is therefore uncertain whether the vivid epoxygenation is native or whether it is induced by drugs or by other means. The important conclusion for the present study is that monkey liver did not contain a prominent fatty acid \(\omega\)-hydroxylase, although small amounts of this enzyme might have been masked by the many other prominent oxygenation reactions in the liver. Nevertheless, P-450\(_{18}\) might be specific for specialized tissues like the seminal vesicles.

The hepatic and renal cortical epoxygenases are more prone to accept signals from the adjacent tissue and they might function in specialized tissues as epoxygenases.
to oxygenate distal double bonds than double bonds close to the carboxyl group of polyunsaturated fatty acids (7, 8). Little is known about the regiospecificity of the various isozymes. Indeed, some isozymes (rat P-450DP, rabbit P-450LM, and human P-450 AA) seem to oxygenate all four double bonds of arachidonic acid to a similar extent (10, 33). There are reports, which indicate selective oxygenation of double bonds, e.g. the 11(12)-double bond of arachidonic acid in renal cortex (34), but the results are in conflict with other studies (7, 9). It may therefore be correct to conclude that a position-specific fatty acid epoxygenase has not been described. If these enzymes do exist, it seems likely to find them in specialized tissues. As regards the epoxidation of eicosatetraenoic acid by microsomes of seminal vesicles, it seems likely that this reaction is catalyzed by a cytochrome P-450 isoyme with at least some double bond specificity. Epoxidation of the other four double bonds of eicosapentaenoic acid was not observed.

It is known that ω3-epoxidation of fatty acids of the (n-3) series occur in hepatic microsomes, although not as an isolated reaction but together with epoxidation of other double bonds as well (34, 35). The apparently specific epoxidation of the ω3 double bond of eicosapentaenoic acid by seminal microsomes is therefore of principal interest. The 17(18)-epoxide is then presumably hydrolyzed enzymically to the vicinal diol, 17,18-dihydroxyeicosatetraenoic acid.

Arachidonic acid is presumably the single most interesting polyunsaturated fatty acid due to its oxidative metabolism to families of biologically active products. The cytochrome P-450 metabolites of arachidonic acid of current interest are epoxyeicosatrienoic acids and 12(R)-HETE. The present study adds 18(R)-HETE and 17(18)-epoxyeicosatetraenoic acid to this list. 18-R-HETE was described by Shi et al. (23) as a metabolite of arachidonic acid by the fungus G. graminis. This is an efficient means of obtaining 18-R-HETE in large amounts and the present study shows that seminal and fungal 18-HETE both have the same configuration (R,R). Shi et al. (23) found that 18-HETE was a poor substrate of fatty acid cyclooxygenase, but possible biological effects of 18(R)-HETE have not been investigated.

G. graminis was found to metabolize eicosapentaenoic acid to 17,18-dihydroxyeicosatetraenoic acid in high yields, presumably via enzymatic hydrolysis of 17(18)-epoxyeicosatetraenoic acid. The seminal microsomes and the fungus thus seem to catalyze the same reactions. This finding lends some support to the hypothesis that the two reactions, ω3-epoxygenation and ω3-hydroxylation, might be catalyzed by the same enzyme. Interestingly, Ortiz de Montellano and his colleagues (5) recently reported that purified lactic acid ω-hydroxylase also catalyzed epoxidation of terminal double bonds. If both ω3 reactions in seminal vesicles are catalyzed by the same enzyme, the geometry of the epoxye should be expected to be R,S. It will be of interest to determine if this is the case. Interestingly, it is already known that 80% of the ω6-epoxide of arachidonic acid, 14(15)-epoxyeicosatrienoic acid, has the R,S configuration (36), when formed by hepatic cytochrome P-450.

Many tissues have been investigated for the presence of fatty acid cyclooxygenase, but a systemic screening for presence of P-450-dependent metabolism of arachidonic acid in different endocrine and genital glands have not been published. The present study shows that fatty acid cyclooxygenase, fatty acid ω3-hydroxylase/epoxygenase, and prostaglandin 19-hydroxylase occur in seminal vesicles. The fact that 19(R)-prostaglandins are the most abundant prostaglandins in primate seminal fluid (22) indicates that they may be of physiological significance, while it is unknown whether 18(R)-HETE or 17,18-dihydroxyeicosatetraenoic acid occur in primate semen. Systematic studies are therefore needed on cytochrome P-450 metabolism of polyunsaturated fatty acid in various other tissues than the liver and the renal cortex and on the presence of cytochrome P-450 metabolites of arachidonic acid and eicosapentaenoic acid in semen and other body fluids.

Acknowledgments—I am grateful to Dr. M. Hamberg for generous help and advice.

REFERENCES


Continued on next page.
18(R)-Hydroxyeicosatetraenoic Acid

**Fig. 1**. Mass spectrum of the 18:0 TMS derivative of 18:0 hydroxyeicosatetraenoic acid methyl ester. A, Mass spectrum of the 18:0 hydroxyeicosatetraenoic acid methyl ester, and the mass spectrum of the 18:0 hydroxyeicosatetraenoic acid methyl ester of the diglyceride (18:1) derivative. B, Relative abundance of the 18:0 hydroxyeicosatetraenoic acid methyl ester of the diglyceride (18:1) derivative. C, Relative abundance of the 18:0 hydroxyeicosatetraenoic acid methyl ester of the diglyceride (18:1) derivative.
18(R)-Hydroxyeicosatetraenoic Acid

Metabolism of arachidonic acid by hepatic microsomes and microsomal membranes.

Metabolism of arachidonic acid by hepatic microsomes and microsomal membranes.

**Fig. 5a:** Mass spectrum of 18,18-dihydroxyeicosatetraenoic acid after hydride-reduction. Me2Si ether methyl ether derivative.

The metabolite eluted in one peak on HPLC (elution volume 73-74 ml). The GC-MS analysis showed the presence of two major compounds, 16-hydroxy-octadecadienoic acid and 11-hydroxy-octadecadienoic acid. The latter was identified as 11-hydroxy-octadecadienoic acid (M+18) and the major compound was shown to be 16-hydroxy-octadecadienoic acid (M+18), after methyl ester derivative. It is shown in A of Fig. 5a, relative ion chromatograms of fragment from the type of 11-hydroxy and 16-hydroxy-octadecadienoic acid are shown as B, i.e. M+18 (M+17) and M+18 (M+17) (M+18) (M+17).

**Fig. 5b:** A, mass spectrum of 16-hydroxy-octadecadienoic acid (Me2Si ether methyl ether derivative). B, relative ion monitoring of some fragments.

**Fig. 6b:** HP-HPLC of metabolites formed from arachidonic acid, PGE, and 18-nor-PGE. Microsomes of Cynomolgus monkeys and P388 A, metabolizing PGE. Arachidonic acid. The paper inserts contained the triolein and cholesterol. The standards were treated with methanol and the corresponding PGE compounds were methylated and analyzed by HP-HPLC. I, II and III designate the methyl ester derivatives of 18-hydroxy, 18-hydroxy-, and 18-hydroxy-PGE, respectively. Metabolism of PGE were methylated and separated by HP-HPLC. I, II and III denote the main compounds as in B.