Isolation and Biosynthesis of 20-Hydroxyprostaglandins $E_1$ and $E_2$ in Ram Seminal Fluid*

(Received for publication, January 6, 1986)

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Ram semen was found to contain 20-hydroxyprostaglandin $E_1$ and 20-hydroxyprostaglandin $E_2$. The relative amounts of the two compounds were almost equal, although ram semen contained at least 10 times more prostaglandin $E_1$ than prostaglandin $E_2$. The accessory genital glands of the ram were analyzed for their capacity to metabolize [1-14C]arachidonic acid to prostaglandins. Biosynthesis of prostaglandins was only found in microsomes of the mucosa of the ampulla of vas deferens and in microsomes of the vesicular glands. Ram vesicular glands and the ampulla of vas deferens were also found to contain the two 20-hydroxylated $E$ prostaglandins. Microsomes of ram vesicular glands and NADPH metabolized exogenous prostaglandin $E_2$ to 20-hydroxyprostaglandin $E_2$ albeit in low yields. Prostaglandin $E_2$ appeared to be a better substrate than prostaglandin $E_1$. Microsomes of human seminal vesicles and NADPH metabolized exogenous prostaglandin $E_2$ to 19-hydroxyprostaglandin $E_2$. The results show that 19- and 20-hydroxylation of prostaglandins occurs in human and ram seminal vesicles, respectively, and possibly also in the ampulla of vas deferens of the ram. The ram and human enzymes specifically hydroxylated the terminal and the penultimate carbon of prostaglandin $E_2$, respectively.

Prostaglandins can be metabolized by $\omega_1$- and $\omega_2$-hydroxylation in the liver, the renal cortex, and the lung of several species (1-3). These reactions are catalyzed by monoxygenases and might be of quantitative importance for the metabolism of prostaglandins (1-4). Hydroxylated prostaglandins may also be of physiological significance. The semen of man and some primates contains a remarkably high concentration of 19-hydroxylated prostaglandins (5-8). In human semen, the concentration of 19-hydroxy-$PGE_2$ is five times higher than that of $PGE_1$ (7). It is likely that the 19-hydroxylated prostaglandins in semen are formed by monoxygenases but the anatomical location of the biosynthesis and the nature of the enzymes have never been determined. 19-Hydroxylated prostaglandins occur in the ejaculate of man after vasectomy (9-11) and the hydroxylation of prostaglandins apparently occurs somewhere in the distal part of the urogenital tract. Ram seminal fluid and seminal fluid of most experimental animals do not contain 19-hydroxylated prostaglandins (8, 12), and 20-hydroxylated prostaglandins have never been described in seminal fluids. In the present report we demonstrate that ram seminal fluid does contain 20-hydroxy-$PGE_1$ and 20-hydroxy-$PGE_2$ in amounts which are only inferior to that of $PGE_1$. Since the 20-hydroxyprostaglandins of ram semen might be biosynthesized in a similar manner as the 19-hydroxylated prostaglandins in seminal fluid of primates, we have studied the anatomical location of these reactions in the ram and report a prostaglandin 20-hydroxylase enzyme in the vesicular glands. The enzyme prefers $PGE_2$ over $PGE_1$ as a substrate and seems to be specific for hydroxylation of the terminal carbons of $PGE_1$ and $PGE_2$. A corresponding enzyme in human seminal vesicles was found to be specific for the hydroxylation of the penultimate carbon of $PGE_2$.

**EXPERIMENTAL PROCEDURES**

**Materials**

[1-14C]Arachidonic acid (59 mCi/mmole), [1-14C]eicosapentaenoic acid (59 mCi/mmole), and [1-14C]PGE$_2$ (58 mCi/mmole) were from the Radiochemical Centre, Amersham, England. PGE$_1$ was from The ONO Pharmaceutical Company, Osaka, Japan. PGE$_3$ and PGE$_5$ were from The Upjohn Co. (courtesy Dr. J. E. Pike).

1-[$\beta^3$H]PGE$_2$, was obtained by biosynthesis (13). Reversed-phase HPLC was performed with equipment as described (13, 14). Cartridges with silica (SepPak) and octadecasilane silica (SepPak/C$_18$) were from Waters Associates. N,O-bis(trimethylsilyl)trifluoroacetamide was obtained from Pierce Chemical Co. Didodecylsodium, a potent inhibitor of cyclooxygenase (15), was from Ciba-Geigy, Basel, Switzerland. Most chemicals and solvents were from Merck. TLC plates, precoated with 0.25-mm silica gel, were from Merck. Radioactivity was determined by liquid scintillation (TriCarb, Packard Instrument Co.) using Ready-Solv HP (Beckman Instruments) as scintillator or by a TLC scanner (Berthold Durnschichtscanner II). Ram seminal fluid was pooled in total volumes of 5-10 ml and stored at $-80^\circ$C. 19-Hydroxy-PGB$_2$, and 19-hydroxy-PGB$_2$ were obtained from human seminal fluid (13). Ram vesicular glands and other accessory genital glands of the ram were obtained at a local slaughterhouse usually within 30 min after death of the animals. The organs were put into ice-cold saline, handled at $+4^\circ$C and used within 1-2 h or stored at $-80^\circ$C for up to a few weeks. Seminal vesicles were also obtained from a 63-year-old man, who underwent surgery for carcinoma of the urinary bladder, and the vesicles were handled in the same way.

**Experiments**

1) **Ram Semen**

5-10 ml of ram semen was added to 10 volumes of acetone and centrifuged or filtered. The acetone extract was evaporated to dryness, extracted on one or two cartridges of octadecasilane silica as described (14) and the prostaglandin fraction was purified by reversed-phase...
HPLC (system B) as described (13, 16). A small amount of \(^{14}C\)-labeled PGE\(_1\) was added to the extract before this HPLC (cf. Ref. 16) and the material, which was more polar than PGE\(_1\), was pooled and treated with alkali, methylated, and purified by reversed-phase HPLC (system A). In some experiments the acetone extract was evaporated and treated with alkali and, after extractive isolation with diethyl ether, the products were methylated and purified by reversed-phase HPLC (system B).

2) Endogenous Prostaglandins in Vascular Glands and in the Ampulla of Vas Deferens

Ram vascular glands were squeezed by hand or by a garlic press and 10 ml of the fluid was processed. The amniotic of vas deferens from 10-15 animals were squeezed by hand and the fluid (8 ml) was processed. The squeezed material was added to 3 volumes of ethanol and centrifuged. After extractive isolation, treatment with alkali, and methylation, the B prostaglandins were separated and analyzed by HPLC and by GC-MS.

3) Metabolism of \(^{4}C\)Arachidonic Acid

Accessory genital glands of the ram (testicles, ampulla of vas deferens, epididymis, vascular glands, and bulbourethral glands) as well as the pelvic urethra (containing the disseminate prostate gland) and the trigonum of the urinary bladder were homogenized and each homogenate was incubated with the radiolabeled substrate (0.25-0.5 \(\times\) 10^6 cpm) for 30 min at 37°C. In other experiments, low speed supernatants (8000 \(\times\) g) were used. Microsomes of ram seminal vesicles were prepared by differential centrifugation (13) and microsomes of the ampulla of vas deferens were prepared in the same way and incubated in a similar manner. The capacity of microsomes from vascular glands and the mucosa of vas deferens to metabolize radiolabelled arachidonic acid was assessed by incubating serial dilutions of the microsomes with the same amount of radioactivity for 3 min at 37°C. After termination with ethanol and extractive isolation, the biosynthesis of prostaglandins was assessed by TLC (using ethyl acetate as eluant).

4) Metabolism of PGE\(_1\) and PGE\(_2\)

(i) Microsomes—Microsomes of ram or human seminal vesicles were prepared in 0.1 M KHPO\(_4\) buffer (pH 7.4) containing 1 mM dicyclofenac sodium but otherwise as described (13). The microsomes (~5 mg of protein) were suspended in 5 ml of the buffer with 0.75 mM dicyclofenac sodium, 1 mM NADPH, and 0.14 mM PGE\(_2\). In some experiments, the incubation was divided into two equal parts: one was incubated for 30 min at 37°C while the other was kept at 0°C, and both were analyzed at regular intervals. The incubations were terminated by addition of 2 volumes each of acetonitrile and ethanol. After centrifugation (20,000 \(\times\) g), the supernatant was evaporated to dryness. The residue was dissolved in methanol/water and centrifuged. The supernatant was analyzed by HPLC.

(ii) Incubation with \(^{14}C\)PGE\(_2\)—Low speed supernatants of ram accessory genital glands were incubated with radiolabeled PGE\(_2\) (5 \(\times\) 10^6 cpm) for 30 min at 37°C with and without addition of 0.5 mM NADPH. Ram seminal microsomes were incubated with the same amount of radiolabeled PGE\(_2\) and 0.5-1 mM NADPH, NADH, or cumene hydroperoxide. The incubations were terminated with ethanol, and after extractive isolation the products were analyzed by TLC.

(iii) Slices—Ram vascular glands were sliced by hand with razor blades. The slices were put in Krebs-Henseleit medium with 50 \(\mu\)M dicyclofenac sodium and preincubated at 37°C for 90 min under carbon dioxide gas and with frequent changes of the incubation medium. The slices were incubated in the same medium but with addition of 0.2 mM PGE\(_2\) for 50 min. In controls, the slices were incubated with medium only and the prostaglandins were added to the medium after the incubation. The incubations were terminated with ethanol and then processed essentially as in point i above.

Derivatization

Methylation was performed with CH\(_3\)N\(_3\) or by reaction with CH\(_2\)\(_2\)/dipropylethylamine/acetanilide (3:1:300; 0.3-0.5 ml) for 15 min at 30°C (13). Silylation with N,O-bis(trimethylsilyl)trifluoroacetamide was performed as described (13). PGB compounds were obtained by treatment with 0.25 M KOH (in methanol or methanol/water for 20 min) and quantified using a molar extinction coefficient of 27,200 (17).

GC-MS Analysis

The mass spectrometric analysis was performed on a Finniggen 4000 quadrupole mass spectrometer equipped with an Inco data system. An open capillary column of fused silica (30-m SPB-1, Supelco, 0.32-mm inner diameter, 0.25-\(\mu\)m phase thickness) was operated isothermally (280°C). Injections were carried out with a falling needle device. Other conditions were: electron energy, 70 eV and emission current, 0.3 A. The instrument was calibrated with PGB\(_1\) so that the ratio of m/z 220 and m/z 502 was approximately 1. C values were determined from the retention times of saturated fatty acid methyl esters; methyl eicosanoate was given the C value of 20.0, etc.

HPLC

The prostaglandins were separated by reversed-phase HPLC (system A: methanol/water/acetic acid, 600:400:5; flow, 1.5 ml/min; system B: methanol/water/acetic acid, 700:300:2; flow, 1.5 ml/min; column: 7.5 \(\times\) 300 mm, Nucleosil-10). An absorbance detector (Kratos Spectroflow 757) was set at 278 nm for detection of PGB compounds eluting from the reversed-phase HPLC column. Fractions were collected every minute.

Ultraviolet Analysis

UV spectra were recorded with a Shimadzu 210A spectrophotometer using ethanol as solvent.

RESULTS

HPLC Analysis

1) Ram Semen

After treatment with alkali and methylation, the PGB compounds of ram semen were separated by reversed-phase HPLC (system B) as shown by Fig. 1. The main prostaglandin in ram semen is PGE\(_1\), as shown by the large peak with the same elution volume as methyl PGB\(_1\) (peak VII in Fig. 1). Peaks I and II had the same elution volumes as methyl 20-hydroxy-PGB\(_2\) and methyl 20-hydroxy-PGB\(_3\) (the identifications is described below), while peaks III, IV, V, and VI had the same elution volumes as PGB\(_1\), methyl PGB\(_2\), methyl \(\Delta\)-17-PGB\(_1\), and methyl PGB\(_3\). The relative amounts of the PGB compounds were, on an average, setting PGB\(_1\) to 100%; 20-hydroxy-PGB\(_2\) 17%; 20-hydroxy-PGB\(_3\), 14%; PGB\(_2\), 9%; PGB\(_3\), 6%; and \(\Delta\)-17-PGB\(_1\), 1%. The elution volumes of the different PGB compounds are summarized in Table I.

![Fig. 1. Separation of PGB compounds by reversed-phase HPLC. Extracts of ram semen were treated with alkali and methylated after extractive isolation of prostaglandins. Materials in peaks I, II, and IV-VII had the same elution volumes as the methyl esters of 20-hydroxy-PGB\(_1\) (I), 20-hydroxy-PGB\(_2\), PGB\(_3\), 2-HPGA, 20-HPGA, and 17-HPGA. Material in peak III had the same elution volume as PGB\(_1\), due to incomplete methylation. The sensitivity of the UV detector was increased 10 and 50 times as indicated. Absorbance units at full scale, 0.4. System: methanol/water/acetic acid, 700:300:2.](image-url)
the large difference in the relative amounts of the two 20-hydroxylated prostaglandins in approximately equal quantities (the actual ratio was 6:4, cf. Fig. 2), the squeezed material from ram vesicular glands contained 8 times more 20-hydroxy-PGB than 20-hydroxy-PGB1. A similar ratio was obtained from the squeezed material from the ampulla of ductus deferens. In both cases, the compounds were identified by GC-MS.

The squeezed material also showed a different ratio between PGB1 and PGB2 than the seminal plasma. For the squeezed seminal material this ratio was 2.4:1. The corresponding ratio for the ampulla of vas deferens ranged from 0.4:1 to 2.5:1, while the ratio for ram seminal fluid averaged 11:1.

3) Biosynthesis of 20-Hydroxy-PGE in Vitro

(i) Supernatant—Low speed supernatant (8000 \times g) of ram seminal fluid was incubated with NADPH and PGE2 either at 37 or 0 °C. After alkali treatment, the products were separated by HPLC as shown in Fig. 3, A and B. The material in peak I of Fig. 3A was identified as 20-hydroxy-PGB1 by its UV spectrum (\(\lambda_{\text{max}} = 278\) nm in ethanol) and by GC-MS, while the material in peak II had the same elution volume as 20-hydroxy-PGB1. Using the endogenous amount of 20-hydroxy-PGE as an internal standard, there appeared to be a 2-fold increase in 20-hydroxy-PGE in the incubation at 37 °C compared with the control. These chromatograms thus indicated that a prostaglandin 20-hydroxylase was present in the low speed supernatant.

(ii) Microsomes—Microsomes of ram vesicular glands, NADPH, and PGE2 were incubated as in point i and the yield of 20-hydroxy-PGE was increased severalfold by incubation at 37 °C as compared to the control on ice. Only traces of the 20-hydroxy metabolite was present if NADPH was omitted from the incubation. To show conclusively that the 20-hydroxylated metabolite was formed in vitro, we incubated mi-

The separation of the polar products of ram seminal fluid by HPLC (system A) after alkali treatment and methylation is shown in Fig. 2. The material in peak I showed \(\lambda_{\text{max}} = 278\) nm in ethanol and it was identified as methyl 20-hydroxy-PGB1 by GC-MS, while the material in peak II was found by the same methods to contain methyl 20-hydroxy-PGB2. The levels of the two 20-hydroxylated PGB compounds in semen increased 10-fold by treatment with alkali and were apparently formed from the corresponding PGE compounds.

The elution volumes of methyl 19-hydroxy- and methyl 20-hydroxy-PGB1, only differed by 1.2 ml on reversed-phase HPLC (system A), and a similar difference was found for the 19- and 20-hydroxylated PGB2 compounds. The 20-hydroxy-PGB compounds were slightly more polar than the 19-hydroxylated ones. Neither the HPLC analysis (cf. Fig. 2) nor the GC-MS analysis showed any traces of 19-hydroxylated PGB compounds in ram semen.

2) Prostaglandins in Ram Vesicular Glands

The inset in Fig. 2 shows separation by HPLC of products formed by alkali treatment of the squeezed material from ram vesicular glands. While the seminal fluid contained the two 20-hydroxylated prostaglandins in approximately equal quantities (the actual ratio was 6:4, cf. Fig. 2), the squeezed material from ram vesicular glands contained 8 times more 20-hydroxy-PGB1 than 20-hydroxy-PGB2. A similar ratio was obtained from the squeezed material from the ampulla of ductus deferens. In both cases, the compounds were identified by GC-MS.

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20-Hydroxyprostaglandins in Ram Seminal Fluid

Crosomes of ram vesicular glands, NADPH, and tetradeterated PGE, for 30 min at 37 °C. The separation of products (after treatment with alkali) is shown in Fig. 3C. Peak I was found to contain 85% tetradeuterated 20-hydroxy-PGB, and 15% of the protium form. In all experiments, the yield of 20-hydroxy-PGE, was low (~0.5%; a few micrograms). Incubation of ram seminal microsomes, NADPH, and PGE, resulted in a small absolute increase in the material with the same elution volume of HPLC as 20-hydroxy-PGB, (after alkali treatment) as compared with the control kept on ice. Taking advantage of the endogenous amount of 20-hydroxy-PGE, as an internal standard, the yield of the PGE, metabolite was found to be very low (~0.05%).

(iii) Incubation with Slices of Ram Vesicular Glands—For unknown reasons, only two out of four series of experiments showed a clearcut (10-fold) increase in the formation of 20-hydroxy-PGE, from exogenous PGE, over the control as judged from the HPLC analysis. PGE, was also used as a substrate, but in this case the increase in the formation of 20-hydroxy-PGE, over that of the control was only 25% and thus insignificant in comparison with the increase in the PGE, metabolite in a parallel incubation with the same slice preparation. In these experiments the identity of the two metabolites was confirmed by GC-MS.

(ii) Incubation with Radiolabeled PGE,—In short, we found no evidence for hydroxylation of radiolabeled PGE, in any of the studied homogenates, low speed supernatants, or fortified microsomal preparations as judged by TLC. In view of the low enzyme activity described above with yields of about 0.5% and the large endogenous amounts of prostaglandins (diluting the specific activity of the radiolabeled substrate) in some tissues, these negative results may not be so surprising. However, in the bulbourethral glands [14C]PGE, was metabolized to [14C]PGF2, (yield 7%) as judged from TLC (RF = 0.38 and 0.20 for the methyl esters of PGE, and PGF2, respectively).

4) Biosynthesis of Prostaglandins by Accessory Genital Glands of the Ram

Significant conversion of radiolabeled arachidonic acid to prostaglandins (mostly PGE, was noticed only in the ampulla of vas deferens and as expected in ram vesicular glands. In the former case, enzyme activity was found in the microsomal fraction of the mucosa. In comparison with microsomes of the vesicular glands, the mucosa showed a much lower enzyme activity/mg of protein (18). From experiments with serial dilutions of microsomes, approximately 25 times more microsomal protein from the mucosa of the ampulla than from vesicular glands were needed to catalyze conversion of 45% of the radiolabeled arachidonic acid to prostaglandins.

5) Human Seminal Vessicles

(i) Endogenous E Prostaglandins—The 100,000 x g supernatant of the vesicles was analyzed for PGB compounds by reversed-phase HPLC (systems A and B) after alkali treatment and methylation. The compounds were identified by their elution volumes and the relative amounts were: 19-hydroxy-PGB, 100%; 19-hydroxy-PGB, 50%; PGB, 8%; and PGB, 12%.

(ii) Biosynthesis of 19-Hydroxy-PGE, in Vitro—Microsomes of human seminal vesicles and NADPH were incubated with PGE, at 37 °C or on ice (control). After alkali treatment and extraction isolation, the products were separated by reversed-phase HPLC as shown in Fig. 4. The formation of material in peak I was increased several fold over the control and peak I was found by GC-MS and UV analysis (λmax = 278 nm in ethanol) to contain 19-hydroxy-PGB, To show conclusively that exogenous PGE, was metabolized in this preparation, the experiment was repeated with tetradeuterated PGE, as a substrate, and in this case tetradeuterated 19-hydroxy-PGE, was obtained and identified after conversion to the corresponding PGB compound. The mass spectra are discussed below.

GC-MS Analysis

All compounds were analyzed as the Me3Si ether and methyl ester derivatives. The signal at m/z 73 gave rise to the base peaks of all the mass spectra. However, the mass spectra (Figs. 5 and 6) were normalized to the most abundant peak above m/z 100.

1) 20-Hydroxy-PGB Compounds

20-Hydroxy-PGB,—The mass spectrum is shown in Fig. 5A and it was obtained from the material in peak II of Fig. 2. The C value was 27.1. Signals were noted at m/z 510 (M*), 495 (M* - 15), 491, 489 (M* - 31), 351 (M* - 159, loss of -(CH3)2OSiMe3), 323 (unidentified, possibly loss of CO from m/z 351), 299, 249 (M* - 261, loss of -CH(OSiMe3) (CH3)OSiMe3), 219, and 147. This mass spectrum was similar with that previously reported for 20-hydroxy-PGB, (1, 19). The UV spectrum and the C value also supported the proposed structure.

20-Hydroxy-PGB,—A mass spectrum of this compound, which was isolated from ram seminal fluid (peak I in Fig. 2) is shown in Fig. 5B. The C value was 27.1. Strong signals were noted at m/z 508 (M*), 493 (M* - 15), 479, 477 (M* - 31), 418 (weak, M* - 90), 349 (M* - 159, loss of C-16-C-20), and 321 (unidentified, possibly loss of CO from m/z 349). In the lower mass range, signals were noted at m/z 247 (M* - 261, loss of C-15-C-20), 147, 129, and 103. The mass spectrum was similar to that previously reported for this compound (20). 20-Hydroxy-PGB, was thus identified by its UV spectrum, the GC-MS analysis, and the C value.

The composite mass spectrum of 20-hydroxy-[3,3,4,4-2H4] PGB, and its protium form is shown in Fig. 5C. This compound was obtained by incubation of tetradeuterated PGE, with microsomes of ram vesicular glands and NADPH. The
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100 200 300 400 500


mass spectrum shows the presence of 15% of the protium form with the signals discussed above while the deuterated compound gave rise to signals, some of which were increased 4 mass units in comparison with the protium form (cf. Ref. 21), e.g. m/z 512 (M⁺), 353 (M⁺ - 159), 325 (M⁺ - 187), and 251 (M⁺ - 261). These and other important fragments are marked in Fig. 5C.

19-Hydroxy-PGB₁ and 19-hydroxy-PGB₂ show mass spectra, which resemble those of the 20-hydroxylated compounds, but they differ in C values (26.2 versus 27.1) and in the relative intensities of many fragments, e.g. m/z 103 (Me₃SiO⁺=CH₂) and 117 (Me₃SiO⁺=CHCH₃; Ref. 1). In the GC-MS analysis of ram semen we could not detect any 19-hydroxylated PGB compounds.

20-Hydroxy-PGB₁ and 20-hydroxy-PGB₂ were also identified in ram vesicular glands and mucosa of the ampulla of vas deferens, in some of the slices experiments, and in incubations with NADPH as mentioned above by complete mass spectra, which were identical with those discussed above. The C values were also 27.1.

19-Hydroxy-PGB Compounds

19-Hydroxy-PGB₁—This compound was identified in the 100,000 × g supernatant of human seminal vesicles after alkali treatment. The C value was 26.2 and the mass spectrum was similar with that previously reported for this compound (19).
DISCUSSION

The finding of 20-hydroxy-PGE, and 20-hydroxy-PGE, in ram semen was the key observation of the present study. This was of interest for two reasons. First, 20-hydroxyprostaglandins had not previously been found in seminal fluid. Second, very little was known about the 19-hydroxylation of seminal prostaglandins in primates and it now appeared to be a simple task to find the location of the 20-hydroxylase, a related enzyme, in the accessory genital glands of the ram. Ram vesicular glands and the ampullae of the vas were found both to contain the cyclooxygenase enzyme and the two 20-hydroxy-PGE compounds and thus appeared to be likely sites of the biosynthesis. The relatively large amount of 20-hydroxy-PGE, in comparison with 20-hydroxy-PGE, in these tissues also indicated that PGE could be the preferred substrate for the hydroxylation.

We found no evidence of biosynthesis of 20-hydroxy-PGE, in a series of initial experiments with incubations of radiolabeled PGE, with homogenates or microsomal preparations of genital glands and different cofactors. Similar negative findings have also been reported with seminal vesicles of primates (3). With the use of a more sensitive technique, the 20-hydroxylase could eventually be detected in ram seminal microsomes, fortified by NADPH, and in some experiments with slices of ram vesicular glands. In both cases, the yield of 20-hydroxy-PGE, from exogenous PGE, was low and PGE, appeared to be metabolized even more slowly.

We have no explanation for the low enzyme activity in vitro. Kelly and co-workers (8) reported that there was no decline in the concentration of 19-hydroxy-PGE, in a series of frequent ejaculates from the stump-tailed monkey. Furthermore, ram semen contained higher concentration of 20-hydroxy-PGE, than PGE, itself. These observations indicated that the hydroxylation of prostaglandins ought to be a rapid process. It appeared possible that the low enzyme activity might be due to inactivation of the enzyme post mortem. Cytochrome P-450 can, for example, be inactivated by lipid peroxidation and by added hydroperoxides (22, 23). The vivid biosynthesis of prostaglandins and hydroperoxides in seminal vesicles post mortem could make the study of the enzyme difficult, not only by diluting the exogenous substrates with endogenous ones but hydroperoxides and other unstable oxygenated metabolites might have deleterious effects upon the enzyme. We therefore used a prostaglandin synthesis inhibitor, diclofenac sodium, in many incubations, but the 20-hydroxylase could be demonstrated without this inhibitor present. Whether the enzyme activity can be substantially increased by the use of other inhibitors or more optimal conditions for the enzymatic reactions remains to be established.

Human semen contains 19(R)-hydroxyprostaglandins and biosynthesis of prostaglandins occurs in human seminal vesicles (24, 25). Our finding that a 19-hydroxylase is present in the microsomal fraction of human seminal vesicles is one of the major findings of the present study. This result is in agreement with several previous reports, which indicated that the seminal vesicles were likely sites for the hydroxylation (8–11), but the enzyme was never detected.

The human and the ram enzymes show a high degree of specificity for hydroxylation of C-20 and C-19, respectively, as judged from our study of the enzymes in vitro and from the hydroxylated E prostaglandins in ram and human seminal fluid. Human seminal fluid has not been reported to contain 20-hydroxyprostaglandins and we were unable to detect any 19-hydroxylated prostaglandins in ram semen. In view of this specificity and the large amounts of hydroxylated prostaglandins in seminal fluid of ram and primates, the 19- and 20-hydroxylases merit further studies.

Little is known about the physiological function of the seminal prostaglandins in reproduction (26). Studies of the possible biological actions of seminal 19-hydroxyprostaglandins may be of interest for several reasons. They are the major seminal prostaglandins in man and certain primates (5–7). Their biosynthesis is decreased in hypogonadal man but could be increased by treatment with testosterone (27). These prostaglandins are also biologically active (8, 28). 19-Hydroxy-PGE, and 19-hydroxy-PGE, are as active as PGE, and PGE, in relaxing spontaneously contracting human uterine muscle strips (8). The natural isomer, 19(R)-hydroxy-PGE, is also more potent than 19(S)-hydroxy-PGE, in suppressing oviducal and uterine activity in the rabbit (28). However, 19-hydroxyprostaglandins only occur in the seminal vesicles of primates and their possible functions in reproduction cannot be studied in any of the common experimental animals. Our finding of 20-hydroxy-PGE compounds in ram semen may be helpful in this respect. Although seminal 19-hydroxyprostaglandins of man and primates and seminal 20-hydroxyprostaglandins of the ram may have different biological functions, a similar role of the latter in ovine reproduction cannot be excluded and might therefore be worth investigating.

Acknowledgments—We thank Dr. Anne-Charlotte Kinn for biological samples, Dr. J. E. Pike of The Upjohn Co. for generous gifts of prostaglandins, and Ann Persson and L. Soderkvist for providing the ram semen.

REFERENCES