

Experimental. The NMR-spectra were recorded on a Varian model A-60 spectrometer.

Deuterations. The composition of the different runs are given in Table 2. The reactions were performed in NMR-tubes kept at 30°C or 60°C.

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Isolation and Identification of Prostaglandins from Ram Seminal Plasma

Prostaglandins and Related Factors 55

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Ram semen was found in an earlier investigation by Bergström *et al.*¹ to contain at least two different factors with smooth muscle stimulating and blood pressure lowering effects. One of these factors showed certain properties similar to those of PGE₁.

Later investigations by Samuelsson² on human seminal plasma led to identification of PGE₁, PGE₂, PGE₃, PGF_{1 α} , and PGF_{2 α} , and more recent studies by Hamberg and Samuelsson^{3,4} resulted in the isolation and determination of the structures of eight additional prostaglandins. These latter compounds are derivatives of the PGE compounds and can be visualized to be formed by hydroxylation at C-19 and/or dehydration involving the hydroxyl group at C-11.

The present report is concerned with the separation and identification of prostaglandins in ram semen and quantitative determination of the components using the procedure described by Bygdeman and Samuelsson^{5,6} for determination of prostaglandin in human seminal plasma.

Methods. Labeled prostaglandins. Tritium labeled prostaglandin E₁ and F_{1 α} synthesized as described earlier^{7,8} were added to the samples in order to follow separation and recovery.

Extraction procedure. Samples of ram semen were obtained by using an artificial vagina and kept at -20° until being processed. To 10 ml of seminal plasma 60 ml of ethanol was added twice and the supernatants were decanted off after centrifugation. After evaporation under reduced pressure and acidification to pH 3 with N hydrochloric acid the solution was extracted three times with ether. The ether extract was washed with water until neutral reaction and then evaporated to dryness under reduced pressure.

Silicic acid chromatography. Silicic acid column was prepared with ethyl acetate: benzene (45:55) using 1 g of silicic acid (Mallinckrodt) activated at 115°. The column was eluted by gradient elution with increasing concentrations of ethyl acetate essentially as described earlier.^{5,6} Aliquots of the fractions were assayed for radioactivity.

Thin layer chromatography. The procedure as described by Gréen and Samuelsson⁹ was used essentially. The glass plates were coated with silica gel (E. Merck AG, Germany) and silver nitrate in the proportion 30:1. Two solvent systems were used: system A VII according to Ånggård, Gréen and Samuelsson¹⁰ and a slightly modified system A II⁹ where the relative methanol concentration was decreased from 35 to 15. PGE₁, PGE₂, PGE₃, PGF_{1 α} , PGF_{2 α} , and PGF_{3 α} were used as reference substances. The substances were detected by spraying with concentrated sulfuric acid and heated for 15 min. For preparative purposes the reference compounds of the PGE type were detected by spraying with 2,4-dinitrophenylhydrazine while the rest of the plate containing the sample to be analyzed was covered. The zones corresponding to the different reference compounds were removed by suction.¹¹ Part of this extract was treated with 0.5 ml of an 2 N ethanolic solution of potassium hydroxide and heated at 50° for 25 min. After centrifugation the UV absorption was measured at 278 μ m.

Gas-liquid chromatography. F & M Biomedical Gas Chromatograph model 400 was used. The material was analyzed after treatment with diazomethane and converted to trimethylsilyl ether derivatives essentially as described before.^{5,6} Hi Eff-8B (Applied Science Lab.) 0.5 % was applied on 100–120 mesh acid washed and silanized Gas-Chrom P. Column temperature 195°.

Mass spectrometry. The instrument described by Ryhage¹² was used.

Results and discussion. Before extraction tritium labeled PGE₁ and PGF_{1 α} were added to the ram semen. After extraction the material was separated by chromatography on silicic acid using a gradient elution with increasing concentration of ethyl acetate in benzene. Two peaks of radioactivity were found corresponding to the added labeled PGE₁ and PGF_{1 α} .

Separation and identification of compounds of the PGE type. The materials present in the first peak were further separated on TLC using solvent system A VII with PGE₁, PGE₂, and PGE₃ as refer-

ence compounds. After charring with concentrated sulfuric acid, 4 spots were visible, one moving with the front and the three others corresponding to the reference compounds. With material from another experiment only the reference compounds were developed with 2,4-dinitrophenylhydrazine while the rest of the plate was covered. The zones corresponding to the reference compounds were extracted with ethanol by suction. After treatment with alkali the three zones corresponding to PGE₁, PGE₂, and PGE₃ gave UV absorption with maximum at 278 μ m. In another experiment the material present in the PGE₁ and PGE₂ zones were first treated with alkali and after extraction with ether converted to methyl esters with diazomethane. The mass spectra recorded on these derivatives were indistinguishable from those obtained for the methyl esters of PGE₁-278 and PGE₂-278, respectively.

Separation and identification of compounds of the PGF types. An aliquot of the material present in the second peak from the separation on the silicic acid column corresponding to the labeled PGF_{1 α} was further separated on TLC using a modified solvent system A II. After treatment with sulfuric acid three spots were visible. One of them moved with the front, the other two corresponded to the reference substances used PGF_{1 α} and PGF_{2 α} . Further identification was achieved by gas-liquid chromatography. The PGF peak from the silicic acid chromatography was treated with diazomethane and converted to trimethyl-silyl ether derivatives. Two peaks appeared with the same retention times as PGF_{1 α} and PGF_{2 α} treated in the same way.

Concentration of PGE₁, PGE₂, PGE₃, PGF_{1 α} , and PGF_{2 α} in ram semen. Quantitative determination of the concentration of the prostaglandin compounds found in ram semen was performed as described earlier by Bygdeman and Samuelsson.^{5,6} The following results were obtained: 28 μ g of PGE₁, 3.2 μ g of PGE₂, 2.0 μ g of PGE₃, 5 μ g of PGF_{1 α} , and 2.3 μ g of PGF_{2 α} per ml ram semen.

Absence of 19-hydroxylated prostaglandins. Hamberg and Samuelsson^{3,4} found considerable amounts of 19-hydroxylated prostaglandins in human seminal fluid. A comparative study of ram semen has not yet been performed. A pool of ram seminal plasma (25 ml) was therefore extracted and fractionated by silicic acid chromatography as described earlier.^{3,4}

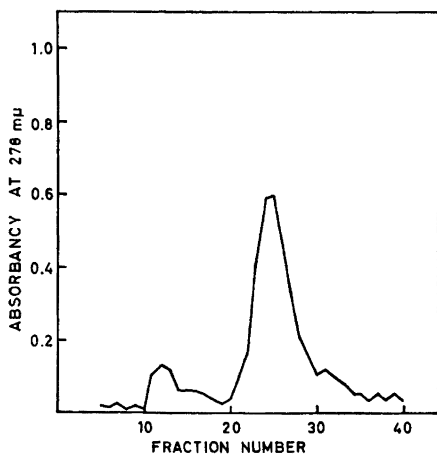


Fig. 1. Separation of aqueous ethanol extract of ram seminal plasma by silicic acid chromatography. Column 5 g. Fraction 15 ml. Ethyl acetate:benzene was used as the eluent in the following ratios: Fractions 1 to 10, 20:80; Fractions 11 to 20, 30:70; Fractions 21 to 30, 60:40; Fractions 31 to 40, 80:20.

After treatment with alkali (0.5 NaOH) and measuring the ultraviolet absorption at 278 $m\mu$ only one peak was found corresponding to the PGE-compounds. No or only slight ultraviolet absorption was found in the fractions eluted with ethyl acetate:benzene, 30:70, and 80:20, indicating the absence of 19-hydroxylated prostaglandins in ram semen (Fig. 1).

These studies therefore show that ram seminal plasma as human contains five prostaglandins PGE₁, PGE₂, PGE₃, PGF_{1 α} , and PGF_{2 α} although the concentration of PGE₂ was lower than in human semen. In difference to human semen 19-hydroxylated prostaglandins could not be demonstrated in ram semen.

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On the Crystal Structure of Bis-L-histidine-copper(II) Dinitrate Dihydrate

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An investigation of the crystal structure of biochemically interesting metal complexes has been started in conjunction with the Department of Biochemistry at this University. In this communication the preliminary results of a crystal structure determination of the bis-L-histidine-copper(II) dinitrate dihydrate are presented.

The crystals were prepared according to a method described by Valladas-Dubois,¹ whereby a concentrated solution of CuSO₄ was mixed with a concentrated solution of L-histidine so as to give a Cu(II):histidine molar ratio of 1:2. A large excess of solid NaNO₃ was dissolved in the resulting solu-