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Note

Thick-layer chromatography for the isolation of 19-hydroxy-prostaglandins from total lipids

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Recently there has been widespread interest in prostaglandins, much of this stemming from their implication in the reproductive processes[1]. The determination of prostaglandins is of clinical interest since it has been suggested that prostaglandins are of importance for a normal fertility[2]. Human seminal fluid has earlier been shown to contain thirteen different prostaglandins[3]. Green *et al.*[4] have developed a resin extraction method for isolation of prostaglandins. Extraction of prostaglandins from human seminal fluid with diethyl ether is a commonly used and accepted method[5]. But only 69.4% ± 2.1 of the 19-hydroxy-prostaglandins are recovered with the ether extraction method[5].

In this paper, a method is described for the extraction of 19-hydroxy-prostaglandins from human semen and their separation from total lipids by thick-layer chromatography.

MATERIALS AND METHODS

Reference compounds

The 19-hydroxy-prostaglandin A_1 (19-OH-PGA_1), 19-hydroxy-prostaglandin A_2 (19-OH-PGA_2), 19-hydroxy-prostaglandin B_1 (19-OH-PGB_1) and 19-hydroxy-prostaglandin B_2 (19-OH-PGB_2) were supplied through the courtesy of Dr. John E. Pike, The Upjohn Company, U.S.A.

Semen samples

The semen used in this investigation was obtained from men (ages 20–25) submitting samples as volunteers. Semen samples were obtained by masturbation.

Extraction of lipids

All reagents were of high or analytical grade. Solvents were re-distilled. Seven milliliters of chloroform–methanol (2:1) were added to 3 ml of semen and shaken well. The contents were centrifuged after 2 h and the lipids extracted by a method described earlier[6]. The nitrogen atmosphere was used at various procedures. The lipid sample was dissolved in the least amount of diethyl ether and kept at 4°.

Preparation of thick-layer plates

Silica gel G, which had been washed once with warm distilled diethyl ether

and subsequently dried at 40° for 6 h in an oven, was used for coating the chromatoplates. Plates measuring 20 × 20 × 0.4 cm were coated with silica gel G at a thickness of 0.2 cm. The plates were dried at 25° for 16 h, heated in an oven at 110° for 30 min and stored over anhydrous silica gel[7].

Separation of standards

The prostaglandin standards and lipid standards in diethyl ether were spotted in volumes of 5–10 μl on the starting-line 2 cm from the edge of the plate and development was carried out at 20° in a chamber with chloroform–benzene (3:2). The solvent was allowed to ascend 15 cm from the starting point. Staining of spots was effected by charring with concentrated sulfuric acid containing 1% potassium dichromate. The sequence of separation is: Total phospholipids, PGE, PGF, 19-OH-PGA$_1$, 19-OH-PGA$_2$, 19-OH-PGB$_1$, and 19-OH-PGB$_2$ (R_F = 0.0); free fatty acids (R_F = 0.21); monoglycerides + diglycerides (R_F = 0.38); cholesterol (R_F = 0.47); triglycerides (R_F = 0.67); cholesteryl esters (R_F = 0.90); hydrocarbons (R_F = 1.0). In another chromatoplate, the above prostaglandin standards and lipid standards were developed in 50% methanol. In this case the prostaglandins moved to the solvent front while the phospholipids did not move from the spot.

Isolation of 19-hydroxy-prostaglandins from total lipids

The lipid sample in diethyl ether was spotted in volumes of 25–30 μl on the starting line 2 cm from both edges of the plate. The chromatoplate was developed in the first direction with chloroform–benzene (3:2) and dried; in the second direction, the chromatoplate was developed with 50% methanol. 19-OH-PGA$_1$, 19-OH-PGA$_2$, 19-OH-PGB$_1$, 19-OH-PGB$_2$, PGE and PGF moved with the solvent front. In this way, the prostaglandins were separated from total lipids. The chromatoplate was dried in a nitrogen atmosphere. The prostaglandin spot on the chromatoplate was identified under UV light[8,9]. The spot was scraped off into a small beaker. Elution was carried out by shaking the silica gel with methanol. After filtering off the silica gel with a very small sintered glass funnel, the methanol was dried at a low temperature under a nitrogen atmosphere. The dry residue was kept in the least amount of ethanol at 4° for further analyses of prostaglandins. The prostaglandins were separated by thin-layer chromatography and estimated; the method was described elsewhere[2,5]. The results are presented in Table II.

RESULTS AND DISCUSSION

Separation and recovery of PGE$_2$, PGF$_{2\alpha}$, 19-OH-PGA$_1$, 19-OH-PGA$_2$, 19-OH-PGB$_1$, and 19-OH-PGB$_2$ by thick-layer chromatography is shown in Table I. Rouser *et al.*[10] show that great care must be exercised in thin-layer chromatography of lipids, because of the wide variations in their migration encountered, depending on the amount and type of gel preparation and on the methods of activating and cooling the plates. Cleaning of the plates of silica gel is also of prime importance[11].

Discussion of extraction procedures for prostaglandins has appeared in several articles[5,8,12]. A number of reports make it clear that the ease of extraction of the prostaglandin decreases in the order of the series F, B, A and E. The present method has the advantage that the presence of non-biologically active substances are excluded.

TABLE I

RECOVERY IN EXTRACTION OF PROSTAGLANDINS IN PERCENTAGE AMOUNTS

Prostaglandins	*Recovery in standard**	*Recovery in human semen***
19-OH-PGA_1	98–100	98–100
19-OH-PGA_2	96–100	95–100
19-OH-PGB_1	99–100	98–100
19-OH-PGB_2	98–100	98–100
PGE_2	95– 99	94– 99
$PGF_{2\alpha}$	96– 99	95– 98

* Data are from six mixtures.
** Data are from four samples to which the standard was added.

Numerous solvent mixtures are tested as developing solvents in an attempt to separate the prostaglandins from other lipid components of human semen by thick-layer chromatography. Thick-layer chromatography is used so that large amounts of samples can be spotted on chromatoplates. The prostaglandins are first extracted from the seminal fluid by thick-layer chromatography and then subjected to group separation by thin-layer chromatography. The results are recorded in Table II. Table II shows the amount of various prostaglandins which are present in human semen. It is noted that about 61 μg/ml of 19-hydroxy-prostaglandins are present. What the role of 19-hydroxy-prostaglandins is in semen remains to be answered.

TABLE II

COMPOSITION OF HUMAN SEMEN PROSTAGLANDINS BASED ON THIN-LAYER CHROMATOGRAPHY METHOD[2,5]

Prostaglandins	*Mean value ± standard deviation** *(μg/ml)*
PGE_1, PGE_2, PGE_3	41.0 ± 5.0
PGA_1, PGA_2	6.0 ± 2.2
PGB_1, PGB_2	4.5 ± 1.3
19-OH-PGA_1, 19-OH-PGA_2	40.8 ± 4.5
19-OH-PGB_1, 19-OH-PGB_2	20.2 ± 3.0
$PGF_{1\alpha}$, $PGF_{2\alpha}$	30.5 ± 4.5

* Data are the average of seven semen samples.

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