Occurrence of Prostaglandins in Fish Testis

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Summary

The qualitative surveys of prostaglandins in fish testis and semen were carried out by thin-layer chromatography, UV absorption spectra, gas-liquid chromatography and bioassay. Prostaglandins were detected as follows: Prostaglandin E_2 in the testis of flounder *Paralichthys olivaceus*, prostaglandin $F_{1\alpha}$ in the semen of chum salmon *Oncorhynchus keta*, and prostaglandin E_2 and $F_{2\alpha}$ in the testis of bluefin tuna *Thunnus thynnus*. The occurrence of prostaglandins was proved in fish testis and semen for the first time, as far as the authors are aware.

In the early 1930's Goldblatt (1) and von Euler (2, 3, 4) discovered respectively that the extract from human semen or sheep vesicular gland contained substances which stimulate the smooth muscle and lower the blood pressure, and later von Euler gave the name prostaglandin (PG) to this substance (3). The PGs were isolated in crystalline form from the vesicular glands of sheep by Bergström and Sjövall (5), and then their chemical formulas were determined. The physiological and pharmacological roles of PGs in mammals have been studied, and they are known to be a family of substances showing a wide diversity of biological effects.

It has become apparent that the presence of PGs are not confined to the male genital organ but that they are found to be widely distributed in various tissues (6, 7), but we have very little information on marine animals. Suzuki and Vogt (8) reported that so called *darmstoff* from frog intestine was identical as PGs, and remarkably high concentrations of a PG-isomer were found in gorgonian *Plexaura homomalla* by Weinheimer and Spraggins (9). The accumulative activities of tritiated PGs in some tissues of marine animals were recently investigated by Bito (10). When the physiological importance of PGs in mammals is taken into consideration from the facts described above, it should seem that PGs are widely distributed not only in higher animals but also in lower animals. To elucidate the occurrence and the biological significance of PGs in relation to essential fatty acids and the reproduction in marine animals, the present authors have made a survey of PGs in fish testis and semen as a first step.

Experimental

Biological Materials

Testis (1500 g) of flounder (*Paralichthys olivaceus*), or bluefin tuna (*Thunnus thynnus*), and semen (680 g) of chum salmon (*Oncorhynchus keta*) were used as the materials in this experiments. The chum salmon, weighing from 3900 to 7000 g, were caught in the River Tsugaruishi in January, the flounder were captured off Fukushima Prefecture in May, and the bluefin tuna was obtained in the fish market of Shiogama port in June. The materials were stored at –20°C until extraction.

Reference Prostaglandins and Chemicals

Authentic PGs were sent from the Upjohn Company and Ono Pharmaceutical Co., Ltd. Silicic acid (Mallinckrodt) and Silica Gel H (Merck) were also obtained from commercial sources. In gas-liquid chromatography, the liquid phase and the solid supports were obtained from Applied Science Laboratories. All other chemicals were the best grades available from Tokyo Kasei Co., Ltd.

Assay for Flounder Testis

The ether extracts were obtained from the testis of flounder according to the method of Samuelsson (11). The extracts were evaporated to dryness under nitrogen, dissolved in benzene, placed on a 17.0×3.1 cm column containing 50 g silicic acid (100 mesh). The solvents employed were as follows; 5×100 ml benzene — ethyl acetate (7:3, v/v); 6×100 ml benzene — ethyl acetate (4:6, v/v); 9×100 ml benzene — ethyl acetate (2:8, v/v); twenty fractions were collected in total. After evaporation the residues of each fraction were further fractionated by preparative thin-layer chromatography using system-A in Table 1 (12, 13). The silica gel of the zone corresponding to R_F 0.00–0.05 was scrapped off with a spatula and extracted with ethanol. Five per cent phosphomolybdic acid in ethanol and 0.1 per cent 2,4-dinitrophenylhydrazine were employed as a spraying reagent.

Assay for Bluefin Tuna Testis and Chum Salmon Semen

The extraction of PGs was made according to the mild modification of the method of Samuelsson (11) and that of Light and Samuelsson (14). The column chromatography was carried out on Silica Gel H (for thin-layer chromatography) which had been activated at 110°C for 1 hr (15). The total lipids in chloroform were applied to 50 g column (17.0×3.1 cm) of silica gel, and the column was eluted with mixtures of methanol in chloroform successively; 3×200 ml chloroform; 3×200 ml chloroform — methanol (9:1, v/v); 4×200 ml chloroform — methanol (65:35, v/v); 2×200 ml chloroform — methanol (2:8, v/v); twelve fractions were obtained in total. The methods of thin-layer chromatography was similar to the case of flounder testis. The PG-areas were located with the aid of iodine vapor and scrapped off, and then extracted with chloroform — methanol (2:1, v/v).

TABLE 1. *Solvent Systems Used in Thin-layer Chromatography*

System	Composition (v/v)
A	Petroleum ether — ether — acetic acid (82: 18: 1)
B	Chloroform — methanol — acetic acid (18: 1: 1)
C	Chloroform — methanol — acetic acid (18: 2: 1)
D	Benzene — dioxane — acetic acid (20: 20: 1)
E	Ethyl acetate — formic acid (75: 4)
F	Cyclohexane — ethyl acetate — acetic acid (6: 4: 1)

Assays of the smooth muscle-stimulating activity were performed as follows; the isolated small intestine from a mouse or a rat was suspended in a 40 ml organ-bath containing oxygenated Tyrode solution at 37°C. The test materials were dissolved in a Tyrode solution and contractions were recorded on smoked kymograph paper (1, 4).

The PG-fraction obtained were methylated with diazomethane, and then were silylated with bis-trimethyl silylacetamide. An aliquot of the silylated methyl ester derivatives (-Me & TMS) was dissolved in ether and was analyzed on a stainless steel column (2 m×3 mm) packed with 3 per cent OV-I on 100–120 mesh Gas Chrom Q or 3 per cent CHDMS on 100–120 mesh Gas Chrom Q (16) using a flame ionization detector (Hitachi gas chromatography model 063). For the reference n-tetracosane was used as an internal standard.

After an aliquot of the PG-fractions was dissolved in methanol, UV absorption spectra was recorded by Hitachi spectrophotometer model EPS-3 with or without alkali treatment (17).

Results and Discussion

Flounder Testis

The material obtained (0.6 mg) was separated by thin-layer chromatography on Silica Gel H with system-B and system-D. It was observed that the RF values of one spot on the chromatoplates in two solvent systems (0.40 and 0.46, respectively) agreed closely with those obtained with the authentic PGF_2 (Fig. 1).

Chum Salmon Semen

The physiological activity on the isolated small intestine from a rat was faintly found in fraction-9 and fraction-10 of the silica gel chromatography. The concentrate of fraction-9 was developed with the authentic PGE_2 and $PGF_{1\alpha}$ using system-B. The chromatogram obtained was shown in Fig. 1. A spot of the chromatoplate had the same RF value (0.29) as that of the authentic $PGF_{1\alpha}$.

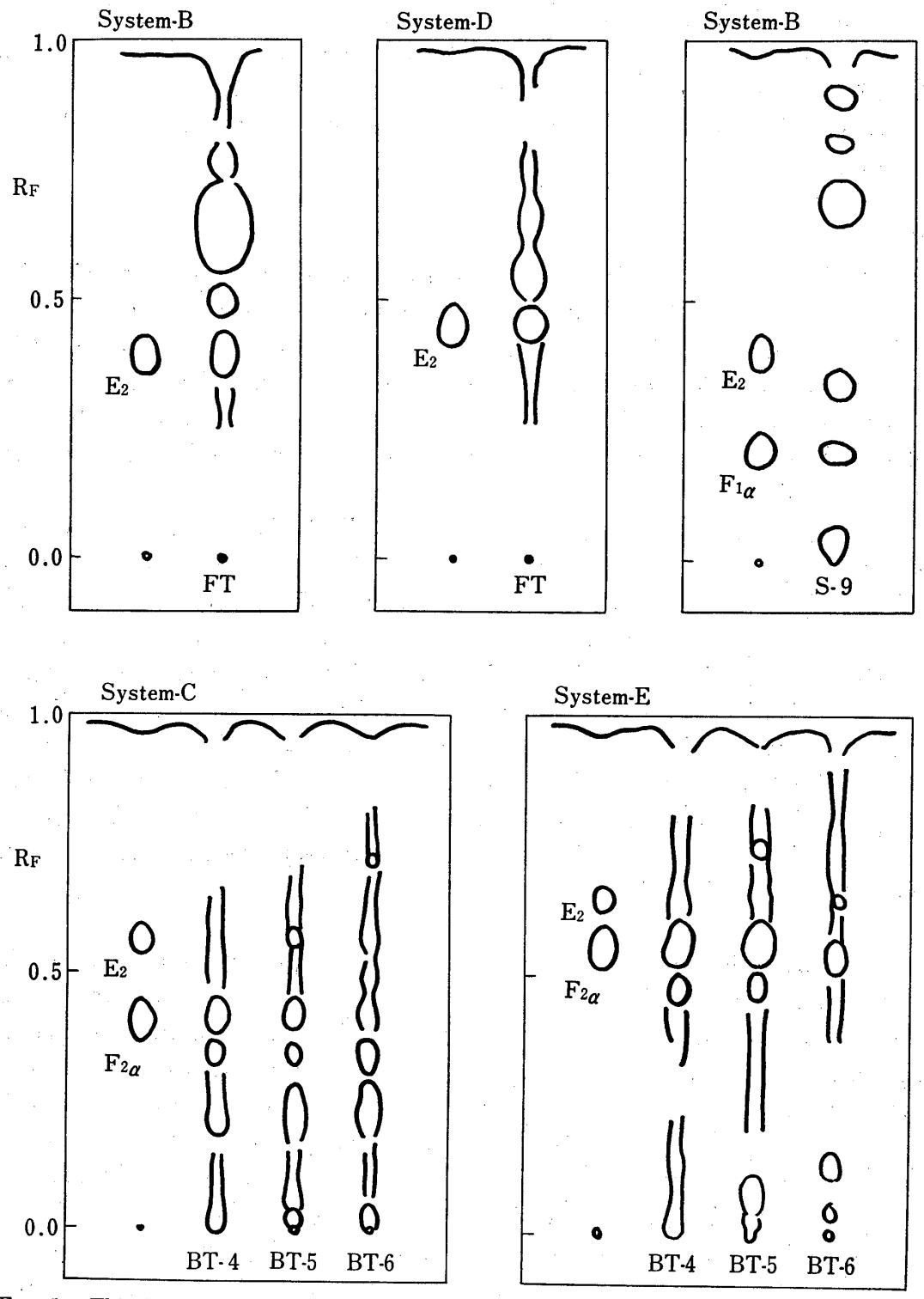

FIG. 1. Thin-layer chromatograms of the authentic PGs and the extracted samples on Silica Gel H. Solvent systems: system-B, system-C, system-D and system-E are shown in TABLE 1.

FT: the PG-fraction of flounder testis S-9: the PG-fraction of chum salmon semen BT-4, -5 and -6: the PG-fraction of bluefin tuna testis of small-scale chromatography (see text).

E_2: Prostaglandin E_2 $F_{1\alpha}$: Prostaglandin $F_{1\alpha}$ $F_{2\alpha}$: Prostanglandin $F_{2\alpha}$

Moreover, fraction-5 was chromatographed using system-F, and showed the characteristic spot at a slightly greater RF value (0.60) than that of the authentic PGA_2 (0.57). A UV absorption maximum of this fraction without alkali treatment was present at about 280 nm. Consequently it seems that this fraction is identical to PGB.

Bluefin Tuna Testis

The fraction-9 from Silica Gel H column chromatography had a physiological activity on the isolated small intestine from a mouse. The active fractions were further purified by small-scale column chromatography on Silica Gel H (for thin-layer chromatography) using the solvent system; 1×5 ml chloroform; 2×5 ml chloroform — methanol (9:1, v/v); 3×5 ml chloroform — methanol (65:35, v/v); 2×5 ml chloroform — methanol (2:8, v/v). In total eight fractions of 5 ml each were collected.

On the chromatoplates the fraction-4, -5 and -6 which were obtained from the small-scale column chromatography showed the same RF's as those of the authentic PGE_2 and $PGF_{2\alpha}$ as shown in Fig. 1 (RF 0.56 for E_2 and 0.42 for $F_{2\alpha}$ in system-C, and RF 0.65 for E_2 and 0.56 for $F_{2\alpha}$ in system-E, respectively). The PGE-fraction treated with alkali showed an absorption maximum at about 280 nm.

The derivatives-Me & TMS of the PGF-fraction from bluefin tuna testis was analyzed by gas-liquid chromatography with the same derivatives of the authentic $PGF_{1\alpha}$ and $PGF_{2\alpha}$ in two liquid phases. The retention time of one peak coincided with that of the authentic $PGF_{2\alpha}$-Me & TMS in two liquid phases. The retention time of the derivatives-Me & TMS relative to n-tetracosane (n-tetracosane retention time=1) were listed in Table 2.

The qualitative survey of PGs in fish testis and semen was carried out by thin-layer chromatography, UV absorption spectra, gas-liquid chromatography and bioassay, and we could obtain positive results within the limit of the present experiments. Hence, it is highly probable that the PGs also occur in fish as in mammals. The semen of human, sheep, monkey, goat and rabbit contained appreciable amounts of PGs, but no PG was detected in semen or male accessory glands of bull, pig, rat, mouse and other animals (18). However, the following facts were reported recently that PGs occur in vesicular gland (15, 19, 20) and testis (15, 21) of rat and in swine testis (22). In view of these reports it might be supposed that the species specificity about the existence of PG was not found in male genital glands of mammals. No comparable data from fish are available, but the results presented here are strengthened by the recent observations described above.

Since most fish is oviparous and have a reproductive mechanism different from mammals, it is possible that a larger amount of PGs is not necessary during the

TABLE 2. *Retention Time of $PGF_{1\alpha}$, $PGF_{2\alpha}$ and the PG-fraction from Bluefin Tuna Testis Relative to n-tetracosane (n-tetracosane retention time=1).*

Derivatives-Me & TMS	(A). 3% OV-I on Gas Chrom Q	(B). 3% CHDMS on Gas Chrom Q
$PGF_{1\alpha}$	2.77	3.98
$PGF_{2\alpha}$	2.54	3.78
PG-fraction from bluefin tuna testis	2.54	3.79

Column conditions

(A): 2 m×3 mm stainless steel column, 3% OV-I on acid washed and silanized Gas Chrom Q (100–120 mesh), column temp., 230°C, detector temp., 280°C, injection temp., 240°C, carrier gas; 50 ml/min nitrogen.

(B): 3% CHDMS on acid washed and silanized Gas Chrom Q (100–120 mesh), column temp., 185°C, detector temp., 280°C, injection temp., 195°C, carrier gas; 50 ml/min nitrogen.

reproductive process in fish. However, it is not deniable that PGs play a significant role in the contraction of the smooth muscle at ejaculation and also in the metabolism of testis in lower animals as well as mammals. It might be thought, moreover, that ovoviviparous fish such as elasmobranches have a higher concentration of PG than teleosts in its genital glands, although there is no evidence on this point. Further experiment is necessary to elucidate fully this interpretation.

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