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Effect of Specific Estrogens on Prostaglandin Synthesis in Aorta and Thrombocytes of Female Pigeons (41064)

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Abstract. The effect of two major natural estrogens (estrone and 17β-estradiol) on prostaglandin biosynthesis from [^{14}C]arachidonic acid in thrombocytes and aorta of female pigeons was compared with that of a male sex hormone (testosterone). In the aorta, 17β-estradiol stimulated the synthesis of 6-keto $PGF_{1\alpha}$ and $PGF_{2\alpha}$ but markedly reduced the synthesis of PGE_2. Estrone on the other hand stimulated the synthesis of PGE_2. Testosterone stimulated the synthesis of all prostaglandins in the aorta. In the thrombocytes, 17β-estradiol decreased aggregatory response to arachidonic acid and synthesis of thromboxane B_2. Estrone on the other hand increased aggregatory response to arachidonic acid. Testosterone decreased the synthesis of thromboxane B_2. These studies have documented markedly different effects of estrone and 17β-estradiol on prostaglandin metabolism in aorta and thrombocytes of female pigeons. Furthermore, it is suggested that testosterone when administered to female pigeons might cause favorable effects through decrease in (a) plasma lipid levels and (b) the synthesis of thromboxane B_2 in thrombocytes.

An increase in thromboembolism and coronary complications has been noted in women following long-term intake of oral contraceptive steroids (1, 2). Studies by Irey *et al.* (3) noted vascular lesions in connection with thrombosis in young women taking oral contraceptives. Because of the role of platelets in thrombosis and atherogenesis (4), it is important to determine whether changes in platelet reactivity and aggregation occur following the intake of oral contraceptive steroids. Bolton *et al.* (5) noted that platelets derived from women on oral contraceptives showed an increased sensitivity to adenosine diphosphate (ADP). Shevde *et al.* (5) failed to notice any effect of oral contraceptives in the response of platelets to ADP, adrenaline, or collagen. Recent studies by Bierenbaum *et al.* (7) showed that susceptible women on oral contraceptives had increased platelet aggregation. The differences in platelet response noted in various studies perhaps might relate to differences in type and dosage of contraceptive steroid mixture used. Systematic studies of the effect of specific natural and synthetic estrogens on (a) platelet aggregation and adhesion (b) platelet and aortic prostaglandin synthesis have not been done. Studies from our laboratory (8) noted that administration of conjugated equine estrogens to atherosclerosis susceptible White Carneau pigeons (female) early in life increased cholesteryl ester accumulation and severity of atherosclerosis in the aorta. In order to elucidate the mechanism of this phenomenon studies were initiated on the effect of specific estrogens on thrombocyte aggregation and synthesis of thromboxane A_2 (proaggregatory material formed by platelets (9)) and prostaglandin synthesis in aorta of female pigeons. In this report, effect of two major natural estrogens (estrone and 17β-estradiol) is compared to that of a male hormone (testosterone) with respect to thrombocyte aggregation and prostaglandin synthesis in aorta and thrombocytes of female pigeons.

Methods. Female White Carneau pigeons (6 months old) were obtained from Palmetto Pigeon Plant, Sumter, South Carolina. Thirty-two pigeons were kept on Purina pigeon chow throughout the experiments, and divided into four groups of eight pigeons. They were weekly injected im with either estrone, 17β-estradiol or testosterone (10 mg/kg in sesame oil) for 9 weeks. Control pigeons were injected with sesame oil. For aggregation studies, blood was drawn from the heart and mixed with 3.8% sodium

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citrate (10) in a ratio of 9:1. The blood was centrifuged at 120*g* at room temperature for 5 min. After stirring the TRP (thrombocyte rich plasma) in one direction with a small wooden stick, the upper layer was removed with a siliconized pipette. Arachidonic acid, 50 μl, solution (in 0.9% NaCl) corresponding to 125, 250, 375, and 400 μg of arachidonic acid was added to 0.45 ml of TRP which was being stirred with a magnetic stirring bar. Change in percentage transmission was noted over a 9-min period with a chronolog aggregometer (Havertown, Pa.). Further dilutions of arachidonic acid were sometimes needed to produce less than maximal aggregation. For the isolation of platelets (10) blood was centrifuged at 130*g* for 4 min at 0°. The platelet-rich plasma was transferred to 15-ml plastic tubes and again centrifuged at 1200*g* for 30 min at 0°. The supernatant plasma was discarded and the platelet pellets were washed with 6 ml of 0.1 *M* phosphate buffer, pH 7.4, followed by centrifugation at 1200*g* for 30 min. Platelet pellets were then suspended in 4 ml of 0.1 *M* phosphate buffer and aliquots were used for experiments involving incubation with [^{14}C]-arachidonic acid.

Aorta was dissected quickly following exsanguination of animals, cleared of fatty tissue, minced, and homogenized with 5 ml of 0.1 *M* phosphate buffer for 2 min with a polytron homogenizer. (12); the homogenate was centrifuged at 1200*g* for 30 min at 0°. The supernatant was used for incubation with [^{14}C]arachidonic acid.

Platelet suspension (1.0 ml) and aortic homogenate (1.0 ml) were incubated with [^{14}C]arachidonic acid (sp act 50 mCi/m*M* from New England Nuclear, Boston, Mass.) for 5 and 10 min, respectively. The reaction was terminated with 20 cc of chloroform/methanol (2:1, v/v) and the mixture was changed to pH 3 with 1 N citric acid. Following the addition of carrier PGE_2, $PGF_{2\alpha}$, thromboxane B_2, and 6-keto $PGF_{1\alpha}$ (courtesy of Dr. J. Pike, Upjohn Co., Kalamazoo, Mich.), 2 ml of 0.9% NaCl was added, mixed, and the lower organic layer was collected. The extract was evaporated to a small volume under nitrogen and subjected to thin-layer chromatography. For platelet extracts, a solvent system (13) consisting of ethyl acetate:isooctane:acetic acid:water (90:50:20:100, v/v/v/v) was used (Fig. 1A). For aortic extracts a solvent system (12) consisting of ethyl acetate:isooctane:acetic acid:water (110:20:30:100, v/v/v/v) was used (Fig. 1B). Formation of 6-keto $PGF_{1\alpha}$ and $PGF_{2\alpha}$ is fairly small in pigeon aorta which is in accordance with our previous study (12). The areas corresponding to PGE_2, $PGF_{2\alpha}$ thromboxane B_2, and 6-keto-$PGF_{1\alpha}$ were scraped off into vials and subjected to scintillation counting (14). While 8–12% of added [^{14}C]arachidonic acid was converted to prostaglandin products in aorta, about 14–18% conversion was noted with thrombocytes. The conversion of [^{14}C]arachidonic acid into various prostaglandins was expressed as dpm product formed/milligram protein estimated by Lowry's procedure (15). Plasma cholesterol and triglycerides were determined by the LRC procedure (16).

Significance of differences between control and steroid treated groups was determined with the student's test for difference between means and P' values of <0.05 were considered significant.

Results. Table I shows the changes in body weight, and plasma lipids of various groups of pigeons. As can be seen from the table the pigeons treated with estrone and 17β-estradiol showed a significant reduction in body weights which might be due to a slightly decreased food intake noted in these groups. The plasma cholesterol concentration in 17β-estradiol treated pigeons was significantly higher while the testosterone treated group had a significant reduction in plasma cholesterol. Plasma triglyceride showed a similar difference in response to 17β-estradiol and testosterone.

Figure 2 shows the differences in the aggregation pattern of thrombocytes (in response to arachidonic acid) from various groups of pigeons. While thrombocytes from testosterone treated pigeons showed no significant differences from the control, both estrone and 17β-estradiol treatment changed thrombocyte aggregation markedly. While estrone enhanced thrombocyte

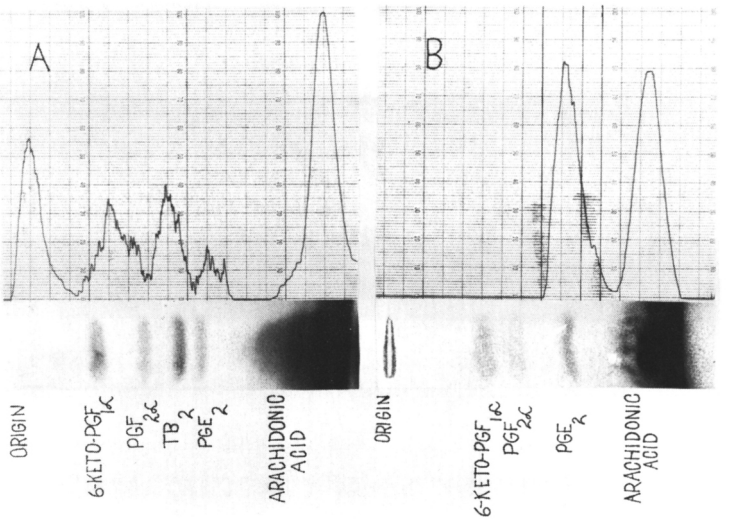

FIG. 1. Radiochromatographic scanning and thin-layer chromatography of the products (following addition of carrier prostaglandins) obtained after incubating [^{14}C]arachidonic acid with pigeon thrombocytes (Fig. 1A) and aorta (Fig. 1B). Chromatographic conditions are described in the text. The plates were sprayed with 10% phosphomolybdic acid in ethanol and heated at 110° for 15 min. Note that the formation of 6-keto $PGF_{1\alpha}$ and $PGF_{2\alpha}$ is fairly small in pigeon aorta (12).

aggregation, 17β-estradiol treatment markedly decreased aggregation. Table II shows the synthesis of various prostaglandins from [^{14}C]arachidonic acid in pigeon thrombocytes. The synthesis of thromboxane B_2 was significantly reduced in 17β-estradiol treated pigeons (correllated with the aggregatory response of their thrombocytes) while no significant difference was seen in estrone treated group. Testosterone decreased the synthesis of thromboxane B_2.

Table III shows the synthesis of prostaglandins in aorta of pigeons from control and steroid treated groups. Administration of 17β-estradiol and testosterone stimulated the synthesis of 6-keto $PGF_{1\alpha}$ (stable product of prostacyclin) and $PGF_{2\alpha}$. However, estrone and 17β-estradiol displayed marked differences in their effect on PGE_2 synthesis. While estrone markedly stimulated synthesis of PGE_2, 17β-estradiol decreased its synthesis. Testosterone caused enhanced synthesis of all prostaglandins in the aorta.

Discussion. This study for the first time has critically examined the effect of two major natural estrogens (estrone and 17β-estradiol) on the synthesis of prostaglandins in thrombocytes and aorta of female pigeons. 17β-Estradiol treated thrombocytes

TABLE I. BODY WEIGHTS AND PLASMA LIPIDS IN PIGEONS (MEAN ± SEM)

Group (n = 8)	Body weights (g)	Plasma lipids (mg%)	
		Cholesterol	Triglycerides
Control	589 ± 10	221 ± 29	109 ± 18
Estrone	543 ± 14*	201 ± 20	173 ± 26
Estradiol	487 ± 23*	452 ± 63**	2860 ± 405**
Testosterone	582 ± 32	201 ± 11**	64 ± 6**

* Significantly different from controls, $P < 0.05$.
** Significantly different from controls, $P < 0.05$.

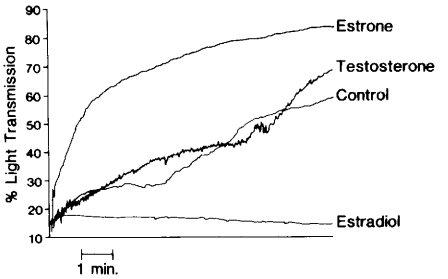

FIG. 2. Thrombocyte aggregation in response to arachidonic acid in control and steroid treated pigeons.

TABLE II. SYNTHESIS OF PROSTAGLANDINS FROM [^{14}C]ARACHIDONIC ACID IN PIGEON THROMBOCYTES (MEAN ± SEM)

Group (n = 5)	Prostaglandin synthesis (dpm/mg protein)		
	$PGF_{2\alpha}$	TB_2	PGE_2
Control	1972 ± 396	7366 ± 1056	1227 ± 2.8
Estrone	1613 ± 600	8014 ± 344	532 ± 130***
Estradiol	1927 ± 487	5707 ± 390*	1541 ± 212
Testosterone	1464 ± 394	4503 ± 706*	991 ± 213

* Significantly different from controls, $P < 0.05$.
** Significantly different from controls, $P < 0.05$.

showed (a) decreased response to arachidonic acid in terms of thrombocyte aggregation and (b) synthesized less thromboxane B_2. Estrone treated thrombocytes on the other hand displayed increased aggregatory response, but the increase in the synthesis of thromboxane B_2 was not statistically significant. Whether the increased aggregatory response of thrombocytes from estrone treated pigeons involves a mechanism other than through the synthesis of thromboxane B_2 remains to be investigated. Testosterone treatment decreased the synthesis of thromboxane B_2 in thrombocytes. In the aorta, 17β-estradiol stimulated the synthesis of 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$, but markedly decreased the synthesis of PGE_2. This is in accordance with the studies by Nam *et al.* (17), who showed that 17β-estradiol stimulated the synthesis of $PGF_{2\alpha}$ in rat uterus. Estrone on the other hand markedly stimulated the synthesis of PGE_2 in aorta. Thus, estrone and 17β-estradiol cause totally different effects in the synthesis of specific prostaglandins in tissues. The effect of testosterone in female pigeons was fairly similar to that of 17β-estradiol.

The effects of estrone and 17β-estradiol on plasma cholesterol and triglycerides were also quite different. While 17β-estradiol caused increases in plasma cholesterol and triglycerides, estrone showed no significant effects on plasma lipids. Thus, the effect of specific estrogens on lipid metabolism is quite complex. As noted by Ferrari and Naito (18) the effect might depend upon the dosage and duration of treatment with specific estrogens. Testosterone treatment in female pigeons decreased plasma cholesterol and triglycerides levels.

In conclusion, our studies have shown that 17β-estradiol, in spite of increasing plasma lipids, causes changes in platelets and aorta prostaglandin synthesis which might favor a decrease in platelet–endothelial interaction (4). Estrone on the other hand, through its enhancing effect on (a) thrombocyte aggregation and (b) increased aortic PGE_2 synthesis (via decreases in cholesteryl ester hydrolysis (12)) might contribute to increased platelet de-

TABLE III. SYNTHESIS OF PROSTAGLANDINS FROM [^{14}C]ARACHIDONIC ACID IN PIGEON AORTA (MEAN ± SEM)

Group (n = 5)	Prostaglandin synthesis (dpm/mg protein)		
	$PGF_{1\alpha}$	$PGF_{2\alpha}$	PGE_2
Control	504 ± 32	2460 ± 424	11,480 ± 444
Estrone	669 ± 156	3465 ± 229	21,220 ± 2662***
Estradiol	728 ± 71	4080 ± 415**	6294 ± 402***
Testosterone	1007 ± 138*	6483 ± 327**	15,837 ± 749***

* Significantly different from control group, $P < 0.05$.
** Significantly different from control group, $P < 0.05$.
*** Significantly different from control group, $P < 0.05$.

position and cholesteryl ester accumulation in the aorta. Testosterone when administered to female pigeons caused beneficial effects by decreasing plasma lipids and decreasing the synthesis of thromboxane B_2. Careful studies of the effect of estrone (the major circulating estrogen (19)) and other synthetic estrogens (used in oral contraceptives) on arterial wall and platelet lipid metabolism are warranted.

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