Measurement of prostaglandins E and F in the uterine tissue and uterine fluid of ovariectomized rats treated with various doses of oestradiol-17 β

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There is now considerable evidence that prostaglandin (PG) F-2 α of uterine origin is the luteolytic substance in several species (for reviews, see Weeks, 1972; Craig, 1975). Although the hormonal control of the synthesis and/or release of PGF-2 α from uterine tissue is not fully understood, both oestrogen and progesterone may be involved. In the ovariectomized ewe PGF concentrations in the uterine tissue increase only when the animal is primed with progesterone and then treated with oestrogen (Caldwell, Tillson, Brock & Speroff, 1972). In the ovariectomized guinea-pig, however, PGF-2 α synthesis can occur without prior treatment with progesterone (Naylor & Poyser, 1975). Although equivalent evidence is not available for other species, Lima, Gimeno, Goldraij, Benders & Gimeno (1971) found that treatment of ovariectomized rats with oestradiol-17 β affected PGF-2 α production only when used in small doses (i.e. 0.1 μ g/day for 3 days); higher doses (i.e. 60 μ g/day for 3 days) were ineffective. In the present investigation, the effects of various doses of oestradiol-17 β on the yield of PGE and PGF from the uterine tissue and fluid of ovariectomized rats were further examined.

Female Wistar rats weighing between 200 and 225 g were laparotomized by midline abdominal incision under ether anaesthesia for ligation of the uterine horns at the uterotubal junction and removal of the ovaries. Each animal was allowed to recover for at least 14 days before further treatment. Five or six rats were allocated to each of 6 groups (A–F): animals in Groups B, C, D, E and F were treated with various doses of oestradiol benzoate (see Table 1), given as two s.c. injections in arachis oil at 36 and 12 h before autopsy, and those in Group A received only the equivalent volume of arachis oil.

The rats were killed by stunning and exsanguination through the carotid arteries. Immediately after death the abdominal cavity was opened, uterine fluid was aspirated with a sterile needle and a syringe and the uterine horns removed. The uterine fluid was then stored at -20°C until extraction for PG measurement by the method described for blood plasma (Sharma, Hibbard, Hamlett & Fitzpatrick, 1973). The uterine horns were freed of fat and blood, chopped and homogenized in ice-cold Krebs buffer with a mechanical mixer emulsifier (Silverson Machines Ltd, U.K.). More Krebs solution was added to give a final tissue concentration of 50 mg/ml. From each tissue homogenate three 200- μ l aliquots were removed and to each was immediately added 1.8 ml 96% ice-cold ethanol. The alcoholic extract was then removed and extracted for PG content by the method of Sharma, Wilson & Pugh (1976). Estimations of PGs were carried out by radioimmunoassay as described by Sharma *et al.* (1973) for PGF-2 α but the method was improved to give sensitivities of about 5 pg/200 μ l sample for PGF and 25 pg/200 μ l for PGE.

The results of the experiments were analysed statistically with Student's *t* test and are shown in Table 1.

Treatment with oestradiol caused a significant increase ($P < 0.05$) in the concentrations of PGE and PGF in the uterine tissue compared with the values in the control animals. The amounts of PGE in the uterine tissue homogenates were substantially lower than those of PGF and although this difference was evident in all the animals studied, the ratio between PGE and PGF was the greatest in the control group.

Table 1 also shows the results of the uterine fluid assays. No fluid could be obtained from the

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Table 1. The content (mean $\pm$ S.E.M.) of prostaglandins E and F in the tissue and luminal fluid of uteri from ovariectomized rats

Group	No. of rats	Dose of oestradiol-17 β (μ g) [†]	Uterine tissue				Uterine fluid			
			Uterine wt (mg)	PGE content (ng/100 mg)	PGF content (ng/100 mg)	PGE:PGF ratio	Uterine fluid vol. (μ l)	PGE content (ng/ml)	PGF content (ng/ml)	PGE:PGF ratio
A	5	0	114.46 $\pm$ 2.69	4.95 $\pm$ 0.83	26.54 $\pm$ 1.38	1:5.36	—	—	—	—
B	6	0.4	188.50 $\pm$ 3.87*	12.54 $\pm$ 0.79*	39.91 $\pm$ 1.71*	1:3.18	—	—	—	—
C	5	2.0	231.84 $\pm$ 11.17*	23.16 $\pm$ 1.13*	57.72 $\pm$ 1.74*	1:2.49	420.00 $\pm$ 25.49	2.83 $\pm$ 0.21	1.18 $\pm$ 0.16	1:0.41
D	6	10.0	244.70 $\pm$ 5.23*	28.02 $\pm$ 1.16*	69.21 $\pm$ 1.89*	1:2.47	508.33 $\pm$ 32.70	3.38 $\pm$ 0.20	1.85 $\pm$ 0.14	1:0.55
E	5	50.0	262.16 $\pm$ 10.36*	26.44 $\pm$ 2.37*	71.80 $\pm$ 1.93	1:3.02	560.00 $\pm$ 48.47	3.58 $\pm$ 0.16	2.06 $\pm$ 0.15	1:0.57
F	5	250.0	281.66 $\pm$ 7.54	19.90 $\pm$ 1.73	70.36 $\pm$ 2.80	1:3.53	550.00 $\pm$ 44.72	3.41 $\pm$ 0.30	1.82 $\pm$ 0.23	1:0.53

* Significantly different from control value, $P < 0.05$.[†] Injected s.c. each day for 2 days.

uteri of untreated rats or those treated with 0.4 μ g oestradiol-17 β . The volume of uterine fluid obtained from rats treated with 2 μ g oestradiol did not differ significantly ($P > 0.05$) from that in rats treated with the higher doses. The concentrations of PGE in uterine fluid were substantially higher than those of PGF.

Lima *et al.* (1971) demonstrated the presence of a PGF-2 α -like material in extracts of uterine tissue of ovariectomized rats treated with oestradiol-17 β : the R_f values of this material on thin-layer chromatograph plates matched those of authentic PGF-2 α but no attempt was made to measure its concentration. An interplay of progesterone and oestrogen cannot be excluded in normal cyclic animals, but considerable evidence is available to suggest that oestrogen treatment alone may effectively increase the concentration of PGF-2 α in uterine tissue. In anoestrous sheep, in which progesterone concentrations in the peripheral circulation are minimal, and in ovariectomized guinea-pigs oestradiol treatment induces a release of PGF- α (Sharma & Fitzpatrick, 1974; Naylor & Poyser, 1975). Castracane & Jordan (1975) have claimed that oestradiol-17 β can stimulate *de novo* synthesis of PGE and PGF in the uteri of ovariectomized rats pretreated with progesterone, but they did not measure PG levels in uterine tissue of animals treated with oestradiol-17 β alone.

Our results, based on radioimmunoassay determinations and by using various dose levels, have demonstrated that oestradiol-17 β alone (i.e. without progesterone pretreatment) is capable of inducing a rise in the PG content of uterine tissue in the ovariectomized rat. The significance of the reversed ratios of PGE and PGF in the tissue and fluid samples is not yet clear. In the rat, PGs of the E type are known to increase capillary permeability (Kaley & Weiner, 1968; Crunkhorn & Willis, 1969), while those of the F type are known to be vasoconstrictors (Pharriss, Cornett & Gutknecht, 1970). The formation of these PGs in the uterine tissue and in the intraluminal environment of the uterus in different proportions may be a mechanism for controlling permeability changes in the uterus at the time of implantation. Studies involving the pontamine blue reaction have demonstrated increased permeability and associated changes in the uterine horns of pregnant mice and rats at about the time of implantation (Finn & McLaren, 1967; Ljungkvist & Nilsson, 1974). In the rat an oestrogen surge occurs on the afternoon of Day 4 of pregnancy (Yoshinaga, Hawkins & Stocker, 1969) and blastocyst attachment takes place on the afternoon of Day 5 (Tachi, Tachi & Lindner, 1970). The differential release of prostaglandins in response to the oestrogen surge in the pregnant animal may

therefore be part of the mechanism of implantation in this species. Further work on these lines is in progress.

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