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Phloretin, A Weak Estrogen and Estrogen Antagonist. (28601)

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Weak estrogens can partially antagonize the uterotrophic effect of strong estrogens. Hisaw *et al.*(1) and Velardo and Sturgis(2) demonstrated that such potent estrogens as estradiol and estrone could be partially prevented by estriol or 16- epiestriol from exerting their full effect upon uterine weight. Nonsteroidal weakly estrogenic compounds such as *p*-hydroxypropiophenone pyridine(3), di-p-hydroxyphenylalkanes and alkenes(4,5), clomiphene(6,7) and dimethylstilbestrol(8) have also been reported to have selective anti-estrogenic activities. The anti-estrogenic properties of dimethylstilbestrol have been extensively investigated and reviewed by Emmens *et al.*(8). At least one compound, 1-(*p*-2-diethyl-aminoethoxyphenyl)-1-phenyl-2*p*-methoxyphenyl ethanol (MER-25) has been reported to inhibit the response of a number of target tissues to several estrogens in several species of animals(10,11,12). This compound, while not demonstrating estrogenicity by the typical cornified vaginal smear or increase in uterine weight with relatively large dosage, nevertheless has shown subtle evidence of estrogenicity(11,12,13,14) and is structurally related to non-steroidal estrogens such as trianisylchlorethylene.

Phloretin (β-(*p*-hydroxyphenyl) 2,4,6-trihydroxy-propiophenone), a compound structurally related to the diphenolic estrogens, has been reported to have weak estrogenic activity(15). It was of interest therefore to investigate this substance for estrogen antagonism as well as for gonadotrophin inhibition and effect on fertility.

Materials and methods. Uterotrophic and anti-estrogenic activities. Immature female Swiss Albino mice weighing 6-8 g were employed in these studies. Estradiol benzoate and phloretin were each dissolved or suspended in sesame oil and administered subcutaneously once daily in volumes of 0.1 ml for 3 days. Animals were sacrificed on the fourth day and their uteri were dissected. The quantity of intrauterine fluid was estimated, the fluid was expressed and the uteri were weighed. *Estrogenic and anti-estrogenic activities.* Mature castrated female Sprague-Dawley rats of approximately 200 g which responded to estradiol benzoate with fully cornified vaginal smears were used in this study. Estradiol benzoate and phloretin were dissolved or suspended in an aqueous vehicle and administered in a single subcutaneous dose. Vaginal smears were made at 24, 48, 64 and 72 hours post treatment and graded as positive for estrogenicity only if cornified or nucleated epithelial cells were present in the absence of leucocytes. *Gonadotrophin inhibition and uterotrophic activity in parabiotic rats.* Immature female litter mate Sprague-Dawley rats were made parabiotic by the method of Bunster and Meyer(16). Estradiol benzoate and phloretin were dissolved or suspended in sesame oil and administered subcutaneously once daily for 10 days to the ovariectomized partner. The animals were sacrificed on the eleventh day and the

TABLE I. Uterine Response to Phloretin in Immature Mice.

Compound	Total dose, mg	No. of mice	Uterus wt, mg	% mice with intrauterine fluid
Control*	—	33	7.4 ± .4†	0
Estradiol benzoate	.00003	35	15.2 ± 1.6	0
	.0001	27	37.9 ± 1.8	81
	.0003	34	48.7 ± 1.4	100
Phloretin	.2	8	8.9 ± 1.1	0
	1.0	14	8.2 ± .5	0
	5.0	14	12.1 ± .7	0
	25.0	9	20.9 ± 1.9	0

* Sesame oil. † Standard error of mean.

uteri and ovaries were removed and weighed. *Gonadotrophin inhibition in intact rats.* Immature male Sprague-Dawley rats weighing 50-60 g were divided into groups of 4 animals. Phloretin dissolved in sesame oil was administered subcutaneously once daily for 10 days. On the day following the last injection the animals were sacrificed and the testes, ventral prostates and seminal vesicles were removed and weighed. *Anti-fertility activity in rats.* Mature female Sprague-Dawley rats weighing 200-250 g were divided into groups of 5-10 animals and mated with fertile males. Day of mating was determined by the presence of sperm in the daily vaginal smear. Estradiol benzoate and phloretin were dissolved or suspended in sesame oil and administered subcutaneously on day 1 through day 4 following mating. On day 13 post mating, a laparotomy was performed and the presence of implantation sites or embryos was determined.

Results and discussion. Phloretin induced a weak uterotrophic response when administered to immature female mice (Table I). A total dose of 5 mg was required to elevate significantly the weight of the uterus above control whereas as little as 0.03 μg estradiol benzoate doubled the uterine size. The highest dose of phloretin employed (25 mg) failed to cause intrauterine fluid accumulations. Total doses of 5 mg or less failed to antagonize significantly the uterotrophic effect of 0.1 μg estradiol benzoate (Table II). Phloretin in total doses of 10 and 25 mg, however, reduced the uterotrophic effect of this estrogen by 43 and 47% respectively. It is interesting that all of the doses of phloretin used reduced the estrogen-induced intrauterine fluid accumulation.

When administered to castrated female rats in doses as high as 25 mg, phloretin failed to alter the castrate state of the vaginal smear or to inhibit the vaginal cornification induced by 0.6 μg estradiol benzoate. A dose of 125 mg of phloretin, however, did induce an estrogenic response in 40% of the animals by 48 hours post injection. This compares with a 100% response when 0.6 μg estradiol benzoate was administered. Phloretin, in a single dose of 125 mg administered concurrently with 0.6 μg estradiol benzoate prevented estrogen-induced fully cornified smears in 60% of the animals at 40 hours post injection. However, at 64 hours after injection of both compounds, fully cornified vaginal smears were obtained from all of the animals.

Administration of estradiol benzoate to the castrated partner of a parabiotic pair of rats produced the anticipated uterotrophic effect (Table III). The contralateral intact partner responded to the estrogen with a reduction in ovarian weight indicating inhibition of the castrated animals' pituitary gonadotrophins. Phloretin was much less potent but did produce the uterotrophic and anti-gonadotrophin effects typical of estrogens.

Weak gonadotrophin inhibition was also observed in immature male rats. Daily subcutaneous administration of 5 mg of phloretin for 10 days reduced the weight of the ventral prostate 34% but the weights of the testes and seminal vesicles were not significantly decreased below that of the controls.

TABLE II. Antagonism by Phloretin of Uterine Effects of Estradiol Benzoate in Immature Mice.

Total dose of compounds				
Phloretin, mg	Estradiol benzoate, μg	No. of mice	Uterine wt, mg	% mice with intrauterine fluid
—	—	29	8.1 ± .5*	0
—	.1	22	38.4 ± 1.9	100
.2	.1	10	33.6 ± 2.9	90
1.0	.1	12	33.3 ± 2.6	75
5.0	.1	13	33.3 ± 2.4	38
10.0	.1	5	25.4 ± 2.6	40
25.0	.1	10	24.3 ± 2.3	60

* Standard error of mean.

TABLE III. Gonadotrophin Inhibition and Uterotrophic Activity in Parabiotic Rats.

Compound	Daily dose	Intact partner Ovaries, mg	Ovariectomized partner Uterus, mg
Control*	—	165.9 ± 8.6†	47.7 ± 5.9
Estradiol benzoate	.001 μg	143.6 ±14.7	52.9 ± 4.9
	.01 "	93.9 ±27.2	64.0 ± 6.8
	.1 "	46.1 ± 5.6	174.3 ± 7.8
Control	—	181.6 ± 9.4	74.4 ±12.2
Phloretin	5 mg	149.5 ± 5.5	79.9 ± 5.6
	25 "	138.0 ±11.4	131.4 ±15.1

* Sesame oil. † Standard error of mean.

Phloretin administered on days 1-4 of pregnancy had weak anti-fertility activity. Only 60% of the animals treated with 25 mg phloretin daily had implantation sites and embryos in the uteri at laparotomy. A similar degree of anti-fertility activity was obtained with the administration of 1 μg of estradiol benzoate. Injection of 10 μg of estradiol benzoate prevented pregnancy in 100% of the rats.

These studies demonstrate that phloretin is a very weak estrogen and relatively large doses are necessary to produce weak antagonism of the effects of estrogen administration. Degree of estrogen antagonism, however, is not related to estrogenic potency of the antagonist. Estriol, a moderately active estrogen, only partially inhibits the effects of more potent estrogens on the mouse or rat uterus(1). The compound 1-(*p*-2-diethylaminoethoxyphenyl)-1-phenyl-2*p*-methoxyphenyl ethanol (MER-25), however, which has little or no estrogenic activity is a good anti-estrogenic substance and completely prevents the expression of the effects of estrogen on several estrogen-sensitive tissues(10,11, 12).

Summary. Phloretin is a very weak estrogen. It induces a uterotrophic effect in immature mice and in ovariectomized rats. Estradiol benzoate-induced uterine growth in the mouse and vaginal cornification in the castrate female rat are partially inhibited by concurrent treatment with large doses of phloretin. This compound is a weak inhibitor of pituitary gonadotrophins in parabiotic female rats and in intact immature male rats. Administration of phloretin to female rats on days 1-4 after mating decreased the number of pregnancies by 40%.

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