At concentrations comparable to that used in our pheromone-production experiments, methoprene hindered survival of immature stages and prevented emergence of F_1 progeny[12,13,14], but did not affect survivorship of reproductively mature adults[12] of these target species. In the present series of experiments, we did not observe any mortality of adult beetles with methoprene treatment.

Dose-response curves obtained in laboratory bioassays with *C. ferrugineus*[6] and *Oryzaephilus* spp.[7,9] indicate that a two-to four-fold increase in pheromone dose above the lower threshold for activity could significantly increase attraction of these species. In preliminary field tests, small traps baited with macrolide pheromones and 4,8-dimethyldecanal were effective in recapturing released *C. ferrugineus* and *T. castaneum*[15]. Addition of methoprene to such attractant baits in a pest-monitoring system could result in trapped living adults functioning as enhanced pheromone sources. If food baits are used[16], prior treatment of the baits with methoprene might enhance species-specific attractiveness, with concomitant inhibition of reproduction of the aggregated populations. Finally, enhanced pheromone production by beetles feeding on methoprene-treated stored products may concentrate populations in the treated product, thereby improving the effectiveness of methoprene as an insect control agent.

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Effect of various doses of catecholestrogens on uterine eosinophilia in the immature rat[1]

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Summary. This paper describes the induction of uterine eosinophilia as well as of deep endometrial edema and increase of uterine wet weight in the immature rat by the catecholestrogens 2-OH-estradiol and 4-OH-estradiol. These effects are thought to be mediated by eosinophils via a specific eosinophil receptor system. 4-OH-estradiol was equipotent with estradiol, whereas the effect of 2-OH-estradiol was significantly weaker.
Key words. Catecholestrogens; uterus; estrogenic responses; uterine eosinophilia.

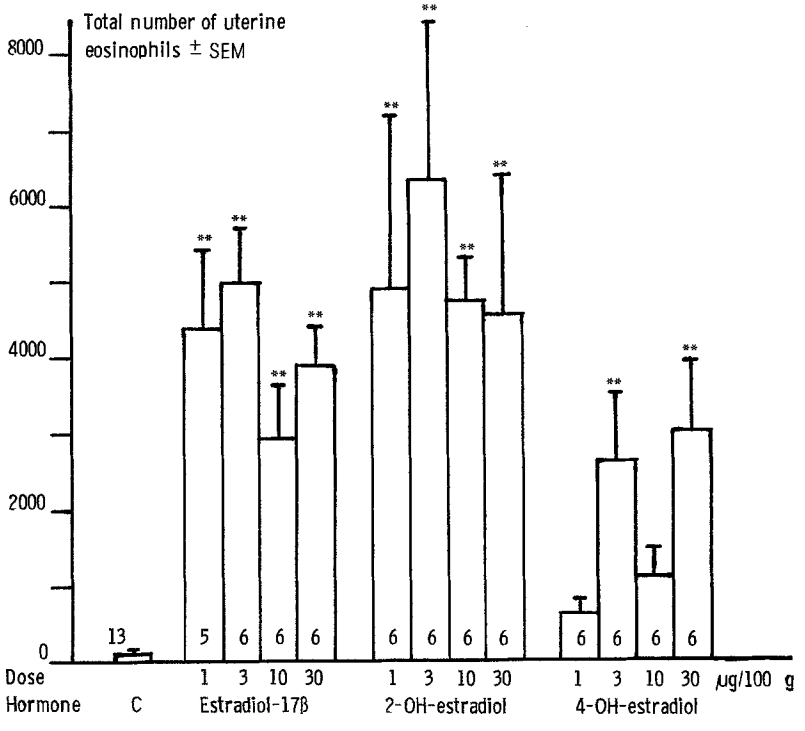
It has been previously shown that estrogens exert their action in the uterus by at least two independent mechanisms: 1) by activation of the genome via the cytosol-nucleus receptor system, leading to induction of several genomic responses (RNA and protein synthesis, morphologic and functional differentiation of target cells)[2], and 2) by an attraction of eosinophil leucocytes to the uterus via the eosinophil-estrogen receptor system postulated by Tchernitchin[3,4], and an induction of several non-genomic responses (edema, increase in vascular permeability and release of histamine in the uterus) under the action of enzymes released in the organ by the eosinophils[4,5]. The hypothesis of multiple and independent mechanisms of estrogen action mediating separate groups of responses is supported by the dissociation of these groups of estrogenic responses, by a number of agents or conditions that selectively interfere with the mechanisms of hormone action involved[4].

The existence of estrogens which exhibit weak estrogenic activity for the induction of some responses but strong for others led us to investigate the estrogenic properties of the 4-OH- and 2-OH-metabolites of estradiol-17-β (E2-17β), whose physiological role is still uncertain. Of these two compounds, called catecholestrogens (CE), 4-OH-estradiol (4-OH-E2) is currently considered to be a relatively strong estrogen with 45% relative binding affinity (RBA) for the cytosol-nucleus receptor, whereas 2-OH-estradiol (2-OH-E2) is thought to be a relatively weak agonist with only 24% RBA and possible partial antagonist properties[6]. The present study reports on the effects of these compounds on uterine eosinophilia, on the eosinophil-mediated edema and on cytosol-nucleus receptor mediated genomic responses.

Materials and methods. 24-day-old female Wistar rats, 45 ± 5 g b.wt, were used. Under ether anesthesia, the rats were i.v. injected with 1, 3, 10 or 30 µg/100 g b.wt. of E2-17β (Sigma Chemical Co., ST. Louis, USA), 2-OH-E2 or 4-OH-E2[7], dissolved in 10% ethanol-saline containing 0.1% ascorbic acid. The control group received the vehicle alone. 6 h after injection the rats were sacrificed, and both uterine horns were excised. After length and wet weight determination, one uterine horn was kept in 0.25 M saccharose for the determination of DNA[8], RNA[8] and protein[9]; the other uterine horn was fixed in 10% neutral formalin for at least 24 h and subsequently histologically processed for the determination of the total number of eosinophils and the number of endometrial and myometrial eosinophils[10,11], and also for evaluation of tissue eosinophil degranulation and deep endometrial edema[12].

Statistics. Uterine eosinophil data for 2-OH-E2, 4-OH-E2 and E2-17β treated animals only were subjected to Tukey's test of additivity, which suggested the square root transformation for further analysis. The Bartlett's test, one way ANOVA and the Student-Newman-Keuls multiple range a posteriori test were performed on square root transformed eosinophil data including the data from the control group, as well as on the data on endometrial edema. Wet weight and biochemical parameters were subjected to log transformation and underwent the F-max test, the Bartlett's test, one way ANOVA and the Student-Newman-Keuls multiple range a posteriori test.

Results. The increase in the number of eosinophils in the uterus induced by various doses of E2-17β, 4-OH-E2 or 2-OH-E2 are shown in the figure. The increases in endometrial and myometrial eosinophils (not shown in figure) follow a similar pattern, although those induced by 3 or 30 µg/100 g b.wt. 2-OH-E2 were significant at $p < 0.05$ only. The percentages of degranulated eosinophils in the uterus were significantly higher after the administration of E2-17β or 4-OH-E2 than after the administration of 2-OH-E2 (χ^2_7=45.4 and 44.1 respectively, $p \ll 0.001$) (table). The increases in uterine wet weight and the appearance of deep endometrial edema induced by the hormones are shown in the table. A parallelism between both responses was observed after a treatment with E2-17β or 4-OH-E2, but not with 2-OH-E2. With 2-OH-E2, deep endometrial edema increased further with higher doses, whereas the increase in uterine wet weight stopped at doses higher than 3 µg/100 g b.wt. The increases in uterine RNA/DNA and protein/DNA ratios after the various treatments are shown in the table. Significant increases in RNA content were observed with the three highest doses of 2-OH-E2, whereas significant increases in protein content were found only with the highest dose of the hormone.



Uterine eosinophilia 6 h after i.v.-injection of various doses of estradiol-17β and catecholestrogens in 24-day-old rats (45 ± 5 g); C, controls; means ± SEM, number of animals per group; ** = $p < 0.01$, as compared with the controls, Student-Newman-Keuls multiple range a posteriori test.

Effect of various doses of estradiol-17β, 2-OH-estradiol and 4-OH-estradiol on RNA: DNA-, protein:DNA ratio, uterine wet weight, deep endometrial edema and the percentage of degranulation of uterine eosinophils in 24-day-old rats (45 ± 5 g b.wt)

		RNA:DNA % of ctrl			prot:DNA % of ctrl			ut ww % of ctrl			d edm ed % of ctrl			% of degran of uterine eosphils[a]
	Dose	n	mean	SEM	n	mean	SEM	n	mean	SEM	n	mean	SEM	n
2-OH-E2	1	6	109ns	15	5	84ns	11	6	120ns	14	6	143*	3.44	6	19.1
	3	6	155*	13	5	106ns	9	6	163**	14	6	147*	5.82	6	41.1
	10	6	158*	15	6	109ns	10	6	152*	14	6	182**	6.40	6	41.8
	30	6	163*	8	6	140*	7	6	158*	19	6	237**	12.30	6	37.0
4-OH-E2	1	6	165*	11	5	123ns	8	6	128ns	8	6	172**	15.10	6	47.7
	3	6	204**	20	4	148**	15	6	153*	15	6	177**	14.20	6	53.3
	10	6	206**	18	5	150**	11	6	232**	20	6	217**	8.32	6	58.2
	30	6	231**	20	4	159**	21	6	167**	14	6	201**	21.30	6	70.5
E2-17β	1	6	179**	22	5	156**	13	6	166**	20	6	173**	15.50	5	58.6
	3	6	172*	18	5	174**	18	6	168**	16	6	173**	21.30	6	67.4
	10	6	154*	8	6	136**	7	6	136ns	7	6	171**	7.25	6	53.4
	30	6	122ns	14	6	129ns	13	6	130ns	14	6	147*	11.30	6	62.5

B.wt, body weight; prot, protein; ut ww, uterine wet weight; d edm ed, deep endometrial edema; degr, degranulation; eosphils, eosinophils; SEM, standard error of the mean; 2-OH-E2, 2-OH-estradiol; 4-OH-E2, 4-OH-estradiol; E2-17β, estradiol-17β; Dose, dose in ug/100 g b.wt; [a]The % of degranulation was calculated from the pooled eosinophils of all animals within each experimental condition; * = $p < 0.05$; ** = $p < 0.01$, ns = $p > 0.05$, as compared to vehicle-injected control rats, Student-Newman-Keuls multiple range a posteriori test. No comparison to controls are performed for the % of degranulation of uterine eosinophils, due to the very low counts of uterine eosinophils in this group.

Discussion. It has been previously shown that estradiol's metabolite, estriol, is more effective in activating the postulated eosinophil receptor system[3] than estradiol. This may indicate that instead of estradiol one of its metabolites is the exact 'key' to this system.

The present report shows that the systemic administration of the short lived 2- and 4-hydroxylated metabolites of E2-17β, compounds that have a higher RBA for the cytosol-nucleus receptor system than estriol[13], also effectively activate the eosinophil receptor system. Since CE are rapidly metabolized[14], and since genomic responses to estrogen follow a time plan in which protein synthesis is maximal after 24 h[15-17], the lack of sustained input after a single injection, the low RBA of 2-OH-E2 and the short time span of 6 h until the animals were sacrificed, may explain the decrease in potency of 2-OH-E2 as compared to 4-OH-E2 and E2-17β in inducing specially the protein-DNA increase. Eosinophil mediated responses were shown to depend on estrogen levels in the blood but not in the uterus[18]. Since the uterus lacks at least 2-hydroxylase[13], it is possible to speculate that, if endogenously formed CE play a role in the uterus under physiological conditions, this would depend on systematic rather than on local concentrations, and CE probably exert their action on circulating eosinophils. It has been shown that E2-17β induces eosinophil degranulation in vivo and in vitro[19] and that enzymes released from degranulating eosinophils (collagenase, beta-glucuronidase arylsulfatase, kathepsin) diffuse to sites distant from the sites where the eosinophils are usually located (in the deep endometrium in the vicinity of the myometrial layer). It has been proposed that any agent or condition increasing or decreasing the degranulation of eosinophils could modify the eosinophil mediated responses, i.e. depolymerization of: small blood vessel collagen fibrils, collagen or mucopolysaccharide[11,19,20].

In this context one could expect that with less degranulation of eosinophils by estrogenic compounds, as has been shown for 2-OH-E2 to occur, there is less diffusion of the above mentioned edema-inducing enzymes, explaining the stronger edematous reaction at the site of eosinophil location (deep stroma) without measurable parallel increase in the wet weight of the whole uterus. The parallelism of deep endometrial edema and uterine wet weight after the administration of E2-17β or 4-OH-E2 on one hand and the divergency observed with 2-OH-E2 on the other hand may alternatively point to different mechanisms of action of these substances. Further studies with a wider dose-range are necessary to elucidate this point.

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Juvenile hormone degradation in brain and corpora cardiaca – corpora allata complex during the last larval instar of *Galleria mellonella* (Lepidoptera, Pyralidae)

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Summary. Time course analysis of juvenile hormone degradation in the brain and the corpora cardiaca–corpora allata complex shows that during the first two days of the last larval instar the juvenile hormone degradation is very low. Starting from the third day up to the seventh day a continuous increase of esterase activity is observed.
Key words. Galleria mellonella; insect brain; corpora cardiaca–corpora allata complex; juvenile hormone degradation.

Organ culture techniques are widely used in insect endocrinology[2]. Short term cultures of corpora allata (CA) have commonly been used for measuring the rate of juvenile hormone (JH) synthesis[3,4]. Fluctuations in synthesis of JH were detected at specific stages of insect development[5]. Recently it was noted that JH produced by CA may not be reliably detected when a standard radiochemical assay is used, owing to JH degradation by JH-esterase associated with the CA[6]. Only one report on JH degradation in CA is available[6]. In the brain no analysis of possible JH degradation was made. In this report an attempt is made to analyze JH degradation in the brain and the corpora cardiaca–corpora allata complex (CCCA) of *Galleria mellonella*. The effect of some inhibitors on JH degradation is presented.

Materials and methods. The *Galleria mellonella* larvae were reared on an artificial diet at 30 °C[7]. The brains and CCCA were dissected from water-anesthetized animals. The organs were removed under MEM medium buffered with 20 mM Hepes/NaOH, pH 6.7 (incubation medium). 1–5 brains or 1–5 CCCA pairs were placed in test tubes and preincubated in 100 µl of incubation medium for 15 min at 30 °C. In the case of hemo-