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.

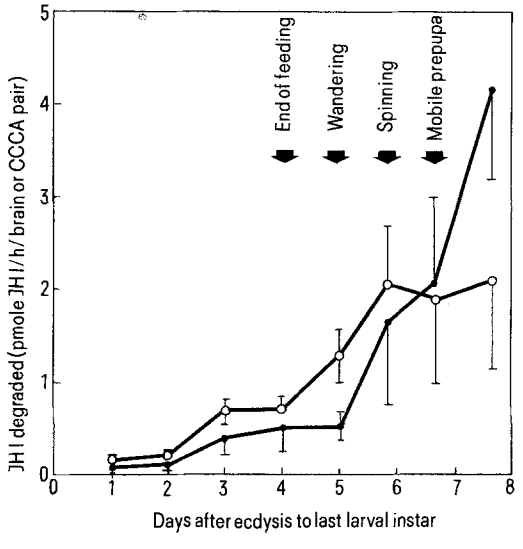
Organ culture techniques are widely used in insect endocrinology[2]. Short term cultures of corpora allata (CA) have commonly been used for measuring the rate of juvenile hormone (JH) synthesis[3,4]. Fluctuations in synthesis of JH were detected at specific stages of insect development[5]. Recently it 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-

lymph, 100 µl of 200–1000-fold diluted hemolymph in incubation medium was used. The enzymatic degradation of JH was initiated by addition of 1 µl of [^{3}H]-JH I (10.5 Ci/mmole, NEN) at a concentration of 4.6×10^{-6} M. The reaction, depending on the enzymatic activity, was continued for 10–60 min at 30 °C. No more than 20% of JH was allowed to convert into product during the course of the reaction. In experiments with inhibitors 1 µl of ethanolic solution of 0.01 M inhibitor was added to 100 µl tissue containing medium and preincubated for 10 min at 30 °C prior to the addition of substrate solution. The inhibitors, α,α,α-trifluoro-4-(4,8-dimethyl-7,8-epoxy-1-nonenyl) acetophenone (TFEA), 1,1,1-trifluoromethyltetradecan-2-one (TFT) and O-ethyl-S-phenyl-phosphoramidothiolate (EPPAT) were a kind gift of Dr B.D. Hammock (University of California, Davis). Controls contained 1 µl of ethanol in place of inhibitor solution. The final concentrations of ethanol, TFEA, TFT and EPPAT in incubation medium were 1%, 0.034%, 0.027%, 0.024%, respectively. The JH degrading activity was measured by means of partition assay[8]. Epoxide hydrolase from the silk gland of *G. mellonella* was partially purified by column chromatography[9]. The epoxide hydrolase activity was analyzed according to Mumby and Hammock[10]. A TLC technique was used to analyze the JH I metabolites. In this case the incubation was followed by extraction with ethyl acetate. The extracted JH I and its metabolites were spotted on silica gel plates and the chromatograms were developed with hexane/ethyl acetate 7:3 v/v. The standards of JH I-acid and JH I-diol were run simultaneously in each plate. The JH I-acid was obtained by enzymatic cleavage of JH I using purified JH esterase[11] and the JH I-diol was prepared by acidic hydrolysis of JH I[12].

Results and discussion. The profile of JH degradation in brain and in CCCA during the last larval instar is shown in the figure. During the first 2 days of the last instar this activity is relatively low: 0.17–0.22 pmoles JH I/h/brain and 0.04–0.08 pmoles JH I/h/pair of CCCA. In the brains 68±8% of JH degrading activity is represented by epoxide hydrolase as judged from TLC analysis.

In CCCA on the other hand, only 5±3% of JH degrading activity corresponds to epoxide hydrolase; the remaining 95±5% of JH degrading activity is due to JH-esterase activity. After the 2nd day of the last instar a continuous increase of JH degrading activity in brains and CCCA is observed. Just before larval-pupal ecdysis in brain and in CCCA the JH-degrading



Juvenile hormone degradation in brain (open circles) and corpora cardiaca–corpora allata complex (closed circles). Each point represents a mean ± SD of 4–18 independent measurements.

The effect of inhibitors on JH degrading activity

Tissue/enzyme	Inhibitor 1×10^{-4} M	Age of larvae VII/1	VII/4
		Remaining activity in percent*	
Brain	EPPAT	49	8.4
	TFEA	5.7	2.4
	TFT	24	10
Corpora cardiaca – corpora allata complex	EPPAT	8.1	0.1
	TFEA	10	1.6
	TFT	7.8	0.4
Hemolymph	EPPAT	2	0.3
	TFEA	5	0.1
	TFT	2	2.1
Epoxide hydrolase fraction**	EPPAT	30	–
	TFEA	0	–
	TFT	0	–

The degradation of JH in the presence or absence of inhibitor was measured by means of partition assay as described in 'materials and methods'. *Each value represents a mean from at least three separate determinations. **Epoxide hydrolase fraction was obtained from silk gland of *Galleria mellonella* by means of Sephacryl S-300 column chromatography[9].

levels are approximately 10 and 100 times higher than on the first day of the last instar in these tissues, respectively. The degrading activity is predominantly due to increase of JH-esterase activity. On the 4th day of the last instar 94±5% of degradation is represented by JH esterase.

The developmental profile of JH degradation in CCCA of *G. mellonella* is analogous to that found in CA of *Manduca sexta*[6]. The increase of JH degrading activity in brain and in CCCA during the last larval instar is concomitant with increasing pupal commitment of these tissues[13]. In the literature two different JH titer profiles during the last larval instar of *G. mellonella* were reported[14,15], although both indicate that 1 day before larval-pupal ecdysis JH titre reaches a small maximum. It was previously found that at this stage of *G. mellonella* development the very intense formation of neurons takes place[13]. Since division of neuroblasts is inhibited by JH, it seems feasible that the tissue-specific JH esterase protects neural tissues against JH circulating in the hemolymph.

The finding that JH degrading activity is present during the whole of the last instar in brain and in CCCA indicates that measurements of JH synthesis in vitro in the absence of inhibitors represent the net result of its synthesis and degradation. It was previously demonstrated that O-ethyl-S-benzoyl-phosphoramidothiolate (BEPAT) at a concentration of 0.1 mM strongly inhibits the JH-esterase activity in CA of *M. sexta* and does not affect the biosynthesis in vitro[6]. We analyzed the susceptibility of JH degrading activity in brain and CCCA to other inhibitors (table). The hemolymph and epoxide hydrolase preparation were used as sources of JH-esterase and epoxide hydrolase activity, respectively. It has been found that the JH-esterase activity present in CCCA on the 4th day of the last instar is nearly completely inhibited by EPPAT, TFT and TFEA. On the other hand the JH degrading activity on the first day of the last instar exhibits a somewhat lower susceptibility to inhibition. A similar pattern of inhibition has been observed in hemolymph. The lower inhibitory effect on degradation of JH on the first day of the last instar can presumably be attributed to a higher contribution of nonspecific esterases or to a higher contribution of epoxide hydrolase. In contrast to CCCA the brain contains significant level of epoxide hydrolase, therefore EPPAT displays relatively moderate inhibitory properties. Conversely TFEA, the trifluoromethylketone derivate containing an epoxide ring, seems to be the strongest inhibitor of JH degradation on the first as well as on the 4th day. The action of TFEA is presumably associated with inhibition of both carboxy esterase and epoxide hydrolase activities.

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Basal plasma corticosterone level after bilateral selective lesions of the olfactory pathways in the rat

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Summary. In comparison to control rats, basal plasma corticosterone level and lactacidemia significantly increased in rats submitted to a bilateral lesion of the lateral olfactory tract and/or the anterior branch of the anterior commissure. Only the lesion of the anterior branch of the anterior commissure induced hyperglycemia; that of the lateral olfactory tract exerted an opposite effect.
Key words. Rat; olfactory pathway selective lesions; plasma corticosterone; lactacidemia; glycemia; adrenal glands.

Olfactory deutoneurons (mitral and tufted cells) located in the olfactory bulb project into the higher nervous centers either directly via the lateral olfactory tract or indirectly via the anterior branch of the anterior commissure and the medial forebrain bundle[1]. Olfactory bulbectomy disturbs emotional reactivity[2,3] and induces either an increase in plasma corticosterone level[4] and adrenal gland weight[5] or the reverse[6]; such results might be due to variations in the extent of lesions, in age, or in the sex (male[4,5], female[6]) of the rats used. The characteristic emotional reactions elicited in sham-operated rats by biologically meaningful odorants (predator or congener odors) are no longer observed after a bilateral section of the lateral olfactory tract, alone[7], or associated with a bilateral lesion of the anterior branch of the anterior commissure[8].

In the latter case, only the medial forebrain bundle remains functional; we have never lesioned it as it is anatomically more diffuse and is involved in various kinds of behavior. On the contrary, after bilateral lesion of the anterior branch of the anterior commissure alone, the reactivity to the odorants slightly increases[8]. Moreover, lateral olfactory tract sections delayed an operant conditioning learning[9] (thirsty rats had to enter and go through a runway for a water reward in less than 6 s). Rats with a lesioned anterior commissure show a decreased ability to exhibit this well-learned behavior again after having been disturbed by an odorous stimulation once during the test (3 or 4 days of conditioning were necessary instead of 1 day at most for sham-operated rats)[9]. Such behavioral alterations are similar to those induced by olfactory bulbectomy[10]. However, the selective influence of each olfactory pathway on the corticoadrenal function remains unknown. The experiment reported here was carried out in order to determine which pathways are responsible for the olfactory bulbectomy effect on corticosterone secretion and on adrenal gland weights.

Materials and methods. Forty male Wistar SPF rats were used. On arrival in the laboratory they were housed in individual cages in the same room and exposed to the natural light-dark cycle in order to obtain experimental conditions identical to those of the behavioral tests[7-9]. Food and water were given ad libitum. The selective olfactory pathway lesions were performed under sodium pentobarbital anesthesia (37.5 mg/kg i.p.) according to the techniques previously described[7,8].

Four groups of 10 rats each were prepared. The control group underwent a sham-operation, i.e. the same procedure (anesthesia, surgery and thus operating stress) as the lesioned animals without lesioning the two olfactory pathways. The bLOT group underwent a bilateral section of the lateral olfactory tract, the bAC group, a bilateral lesion of the anterior branch of the anterior commissure and the bCL group a bilateral section of the lateral olfactory tract, followed two weeks later by a bilateral lesion of the anterior branch of the anterior commissure. Lateral olfactory tract sections were performed on 6-week-old rats, lesions of the anterior branch of the anterior commissure and sham-operations on 8-week-old rats. Then the animals were allowed to recover from surgery during 3 or 4 weeks in order to achieve experimental conditions similar to those of the behavioral tests[7-9]. No anatomical or behavioral recovery occurred during these tests.

When they were 10–11 weeks old the rats were decapitated after 30 s of ether anesthesia, which might slightly increase blood glucose, as reported in the dog[11]. Blood was always collected between 08.00 and 10.00 when the plasma corticosterone level is lowest[12], in order to detect more easily an increase in the corticosterone level induced by the olfactory lesions. Blood samples were taken in order to determine glucose[13] and lactic acid[14] (variations of these parameters being known to be correlated with metabolic disequilibrium, induced in particular by dysfunction of hypothalamo-pituitary-adrenal axis hormones). Plasma

Table 1. Plasma corticosterone, glycemia and lactacidemia in the four groups of rats studied. (Statistical details are given in the text)

Groups	Number of rats	Plasma corticosterone (nmol/l plasma)	Glycemia (mmol/l blood)	Lactacidemia (mmol/l blood)
Control	10	70 ± 31	6.08 ± 0.28	2.52 ± 0.67
bLOT	10	465 ± 112	5.35 ± 0.14	9.74 ± 1.53
bAC	10	1940 ± 148	7.83 ± 0.37	14.91 ± 0.96
bCL	10	500 ± 143	6.62 ± 0.34	6.07 ± 0.99