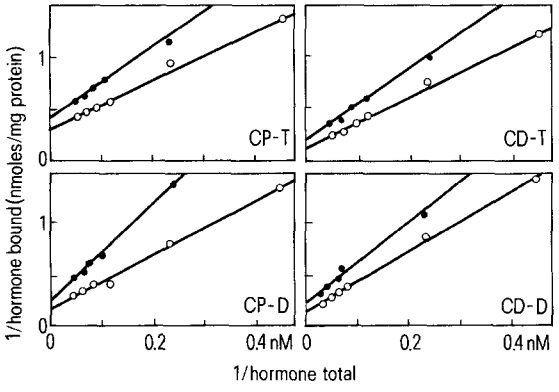

Double reciprocal plots of the data on the binding of testosterone (T) and DHT (D) to the cytosol macromolecules of the caput (CP) and cauda (CD) epididymides, in the presence (−●−) or absence (−○−) of 1 μM CA.

membrane was measured. Protein was estimated according to Lowry et al.[6] and the radioactivity analysed as described[5].

Results and discussion. In the cytosol fractions of the caput and cauda epididymides 1 μM CA inhibited the binding of both ^{3}H-T and ^{3}H-DHT (figure). A double-reciprocal plot of the data showed that the points of intersection of the ordinate by the 2 lines were non-identical, probably indicative of the fact that CA binds to a site on the receptor other than the one occupied by the androgen. Similar results with the cytosol fractions of the ductus deferens, prostate and seminal vesicles showed that in these tissues CA failed to reduce androgen binding (table). On the other hand, there was a marginal increase in hormone binding in the presence of CA.

Whether these results show a qualitative difference between the androgen receptors of the epididymis on the one hand and those of the accessory glands on the other can not be ascertained without additional experimental documentation. However, a gross analysis of the present data does indicate this possibility. According to the currently accepted concepts on steroid hormone action, a saturated hormone-binding to the cytoplasmic receptor protein activates, through an as yet unknown mechanism, the receptor for its migration to the nuclear acceptor sites causing gene activation. The present study shows that an anti-androgen can bind to a site that is not occupied by the androgen and inhibit additional hormone-binding, possibly through a change in conformation of the receptor protein. It may be assumed that this receptor of altered configuration is unable to migrate to the nucleus and interact with the acceptor. However, additional experimental data are required in order to substantiate this hypothesis.

Some of the recent reports on the action of CA upon the reproductive function of male human volunteers[7,8] have documented the following observations. Seminal glyceryl-phosphoryl choline which is a secretory product of the epididymis dropped sharply in all volunteers treated with CA. In vitro sperm migration through mid-cycle cervical mucus (Kremer test) showed that the process was much reduced after CA treatment, again indicating a possible action of the steroid on the epididymis. There were no statistically significant changes in seminal volume, seminal fructose and alkaline and acid phosphatases which are taken as parameters to test the functional integrity of seminal vesicles and prostate[8]. These results, together with the data that we have obtained, emphasize the need for a detailed study of the biological action of CA as a potential male anti-fertility agent and also suggest the use of subhuman primates as better experimental models than the laboratory rodent in such studies.

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In vivo inactivation of denervated corpora allata by precocene II in the bug, *Oncopeltus fasciatus*

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Summary. Transection of the nervous connections between the brain and the corpus allatum (CA) in *Oncopeltus fasciatus* does not alter the susceptibility of the CA to precocene II in vivo.

The discovery by Bowers et al.[2,3] that 2 chromene compounds isolated from plants in the genus *Ageratum* disrupt the development of some hemimetabolous and holometabolous insects, and that these developmental abnormalities can be prevented or reversed by the exogenous application of juvenile hormone (JH), kindled an interest in hormone antagonists as potential 4th-generation insecticides. These chromenes are most active against hemimetabolous species, often causing precocious metamorphosis after exposure of early larval stages or sterility after exposure of adults[3-5]. The most active compound, designated precocene II (P II; 6,7-dimethoxy-2,2-dimethyl-3-chromene), is only effective when the corpus allatum (CA) is synthetically active[6,7]. In addition, treatment of larvae with P II significantly delays molting[4,7]. The inhibition of CA in vitro by P II[8,9], and the histological evidence that P II selectively destroys the parenchymal secretory cells of the CA[10-12], suggest that P II acts directly on the CA rather than on CA control centers in

Table 1. The effect of precocene II (P II) on oogenesis in milkweed bugs (MWB), *Oncopeltus fasciatus,* with a denervated corpora cardiaca-corpus allatum complex (CC-CA)

	N	Treatment	Terminal oocyte ($\bar{x}$ in mm ± SD)	% with eggs in the oviduct
A	8	NCC-aorta cut	1.18 ± 0.032	100
B	13	NCC-aorta cut/P II	0.29 ± 0.051	0
C	21	P II only	0.31 ± 0.116	0
D	4	P II sterilized ♀ MWB implanted with CC-CA from mature ♀ MWB	1.18 ± 0.044	100
E	5	P II sterilized ♀ MWB implanted with CC-CA from mature ♀ MWB/P II	0.051 ± 0.362	60*

A The nervous connections (NCC) and aorta between the brain and the CC-CA were transected at 24 h postecdysis. After feeding for 7 days on milkweed seed and water, the ovaries were dissected from each MWB and the terminal oocytes measured. B The NCC and aorta were transected as in A, and groups of ≤ 5 MWB were confined for 24 h in Petri dishes with a residue of 1 mg P II/dish. The insects were transferred to clean dishes with milkweed seed and water for 7 days, and the terminal oocytes were then measured. C Female MWB 24 h post-ecdysis were sterilized as in B, transferred to clean dishes with milkweed seed and water for 7 days, and the terminal oocytes were then measured. D Female MWB were sterilized as in B; after maintenance for 1 week in clean dishes with milkweed seed and water, a CC-CA from a sexually mature female MWB was implanted in the abdomen of each P II sterilized MWB, and the terminal oocyte was measured 7 days later as in A and B. E Female MWB were sterilized and implanted with a CC-CA as in D, then the insects were retreated with P II as in B, and the terminal oocytes were measured 7 days later. * Reduced number of eggs in the oviduct (1–6 eggs) compared to untreated females (> 10 eggs).

Table 2. The induction of supernumerary nymphs by transplantation of a corpora cardiaca-corpus allatum complex (CC-CA) from young or sexually mature female milkweed bugs (MBW), *Oncopeltus fasciatus,* into 5th instar MWB nymphs 3–5 h post-ecdysis, with or without precocene II (P II) treatment

	N	Treatment		% supernumerary nymphs
		CC-CA host	CC-CA donor	
A	21	5th MWB	mature ♀ MWB	90
B	8	EtOH injection 5th MWB	mature ♀ MWB	88
C	14	P II 5th MWB	mature ♀ MWB	86
D	27	5th MWB	1–2 h ♀ MWB	48
E	11	P II 5th MWB	1–2 h ♀ MWB	0
F	20	5th MWB	P II 7 day ♀ MWB	10

5th instar MWB hosts in treatment (B) were injected with 0.5 µl of 40% ethanol suspension (EtOH injection), and 5th instar MWB hosts in treatments (C) and (E) were injected with 10 µg P II in 0.5 µl of 40% EtOH suspension prior to CC-CA implantation. Donor adult female MWB in treatment (F) were confined at 1 day post-ecdysis for 24 h in Petri dishes with a residue of 1 mg P II/dish (≤ 5MWB/dish), and maintained in clean dishes on milkweed seed and water until dissection.

the brain. We here present the first in vivo demonstration that the nervous connection between the brain and the CA are not necessary for the expression of P II effects in the most sensitive species known, the large milkweed bug, *Oncopeltus fasciatus.*

Methods. The insects used were taken from a culture maintained on milkweed seeds and water ad libitum. Adult female milkweed bugs (MWB) were treated with P II by the contact method[3] using a dose of 1 mg/Petri dish/24 h with not more than 5 individuals per dish. Nymphs were treated with P II by injection of 10 µg in a 40% ethanol suspension into the abdomen with a microcapillary. This dose was previously found sufficient to sterilize adult female milkweed bugs.

All operations were performed under Ringer's solution following brief anesthetization with CO_2. Transection of the nervous connections (nervi corporis cardiaci; NCC) and the aorta in adult female MWB was done through a dorsal incision in the neck membrane, after which the head was secured to the prothorax with wax. Since the corpora cardiaca and corpus allatum in Hemiptera are close together and embedded in the aorta and it has previously been shown[13,14] that sterilization caused by removal of the corpora cardiaca-corpus allatum complex (CC-CA) could be completely reversed by implantation of a CA alone, the entire complex was extirpated for transplantation experiments. In all our experiments the CC-CA was transplanted into the abdomen of the host insect. The activity of CC-CA implanted in P II sterilized female MWB was assessed by dissecting out the ovaries 7 days after implantation, measuring the length of the terminal oocyte, and determining the percent of individuals per treatment which contained eggs in the oviduct. The activity of CC-CA implanted into 3–5-h-old 5th instar MWB was assessed by the percent of individuals per treatment which subsequently molted to perfect supernumerary nymphs[15].

Results and discussion. Cutting the aorta and the nervous connections between the brain and the CA did not inhibit oogenesis in those females surviving 1 week after the operation, but the same operation followed by contact treatment with P II completely inhibited egg development (table 1, A and B). As a cross-check for CA activity in these experiments, some of the CC-CA were recovered from females when the oocytes were measured and transplanted into young 5th instar MWB; the CC-CA from females not treated with P II (A) were active (N = 2), and all but 1 of the CC-CA from P II treated females (B) were inactive (N = 13). The implantation of a CC-CA from a mature female MWB into P II sterilized females completely reversed the effects of P II (D). The same operation as in (D) followed by retreatment with P II allowed the formation of only a few normal eggs (E), indicating again that innervation of the CA is not a prerequesite for the action of P II.

Transplantation of CC-CA from female MWB into 3–5-h-old 5th instar MWB (table 2) produced supernumerary nymphs in about 90% of the operations when mature female MWB were the donors (A and B), but in only 48% of the operations in which young female MWB were the donors (D). Injection of 10 µg P II into nymphs immediately after implantation of a CC-CA did not appreciably inhibit the production of supernumerary nymphs when the donors were mature female MWB (C), but did inhibit the production of supernumerary nymphs when young female MWB were the donors (E). These data further demonstrate that innervation of the CA is not necessary for the inactivation of the CA by P II, and are in agreement with earlier data[9,16] showing that it is easier to prevent the development of the CA in MWB with P II than to cause the degeneration of an active CA. The fact that treatment of nymphal hosts with P II did not significantly reduce the production of supernumerary nymphs when mature females were the donors (table 2, C), whereas some inhibition was demon-

strated in the bioassay using P II sterilized females as hosts (table 1, E), indicates that the cessation of JH synthesis caused by P II is a relatively slow process[6]. The production of only 10% supernumerary nymphs by implantation of CC-CA from P II sterilized female MWB (F) corroborates reports that the damage caused by P II to the CA is irreversible[10,12]. These results indicate that less JH is necessary for the induction of supernumerary nymphs than for stimulation of yolk deposition in adult females. However, 5th instar MWB are only sensitive to implanted CA for about the 1st 13 h after ecdysis[17].

Most of the 5th instar nymphs receiving P II injections required more than the usual 6–7 days to molt. Slama[18] has stressed that the inhibition of growth and the delay of ecdyses are probably due to the antifeedant property of the precocenes. In contrast to the permanent damage done to the CA of sensitive species by P II, it has been our experience that the antifeedant effects of P II are usually temporary; once insects are removed from contact with P II, feeding resumes. It has recently been found that MWB 5th instar nymphs maintained by starvation just below a critical weight will never molt while those that surpass this weight always molt 6–7 days later[19]. Changes in the median neurosecretory cells (MNC) of the brain following P II treatment have been noticed in the MWB[6,20] and there is evidence that the MNC in Hemiptera stimulate food consumption[21]. However, the changes in the MNC of P II treated *Oncopeltus* can be reversed by the application of JH, suggesting that the inhibition of these cells is not permanent and may be due to the absence of a positive feedback from the CA[20].

The results presented here demonstrate a direct action of P II on the CA in vivo rather than on the neural mechanisms of CA regulation. The inactivation of CA by P II in vitro suggests that the parent molecule does not require bioactivation in other tissues. However, since only those CA which are producing JH are susceptible to inactivation it is possible that the cytotoxic effect of P II on the CA results from a specific in situ activation linked to the metabolic activity of the gland.

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Influence of pinealectomy on corticotropin (ACTH)[1]

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Summary. Sensory deprivation produced by removing the eyes and olfactory bulbs in male rats allowed pinealectomy to markedly augment the post-adrenalectomy elevation of ACTH levels. Pineal removal or sensory deprivation separately did not have this effect. Thus, intact sensory input and an intact pineal gland are independently capable of restricting the post-adrenalectomy rise in ACTH levels.

Blinding and anosmia (dual sensory deprivation) in the rat is an established model for pineal-induced suppression of the reproductive system, best reflected in reduced weight of gonadal-dependent structures[3]. The anti-reproductive effect of dual sensory deprivation depends entirely upon an intact pineal gland, with few exceptions[4]. As yet, very little is known about the relationship between blinding-anosmia (BA) and pinealectomy (Px) with respect to the ACTH-adrenal axis.

Materials and methods. 5-week old male Sprague-Dawley rats (Madison, Wisconsin) were randomly divided into groups according to the surgical procedures received: Sh, sham-pinealectomy; Px, pinealectomy[5]; BA, removal of the eyes and olfactory bulbs[6]; and BAPx, combined blinding-anosmia and pinealectomy. The BA group was additionally sham-pinealectomized. Rats were then exposed to a cycle of 12 h light (beginning at 06.00 h) and 12 h dark with food and tap water available until 9 weeks of age, when bilateral adrenalectomy (adx) was performed on a portion of each group. All surgical procedures were done under ether anesthesia by us. Adx animals were given 1% NaCl in water (containing oxytetracycline 1 mg/ml for the 1st 7 days) to drink instead of tap water. 21 days after adx, the rats were sacrificed by guillotine at 07.30 h within 90 sec after the animal room was entered under basal c inditions. At autopsy, all previous surgical procedures were seen to be complete. Ventral prostates and seminal vesicles were excised and weighed wet. Trunk plasma was subjected to radioim-