

Developmental Physiology of Corpora Allata and Accessory Sex Glands in the *Cecropia* Silkmoth

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ABSTRACT The storage of large quantities of juvenile hormone (JH) in adult male *Cecropia* silkmoths is dependent upon the combined activities of two organs: secretion by the corpora allata (CA) and accumulation by the accessory sex gland (ASG). We describe here some aspects of CA-ASG development and physiology which lead to JH storage.

The ASG were found to have completed development and had secreted the luminal materials before eclosion. After day 16 of adult development, the ASG were able to convert JH-I acid to JH-I and store the JH-I. When incubated in vitro, the ASG showed a specificity for the uptake and storage of the three JH acids in the following order: JH-I > JH-II >> JH-III. The CA of male and female larvae secreted JH-I and JH-II when incubated in vitro, whereas adult male CA secreted JH-I acid and JH-II acid. The total equivalent synthetic productivity of adult male CA was found to be considerably greater than that of adult female CA. When cultured in vivo during metamorphosis to the adult in hosts of the opposite sex, the male and female CA maintained their respective pattern of secretory activity. Thus, the CA appear to be programmed to express the sexually dimorphic activity prior to the initiation of adult development.

More than two decades ago the giant silkmoth *Hyalophora cecropia* became the focus of attention in juvenile hormone (JH) research because of the fortuitous discovery by Williams ('56) that abdomens of male moths contain large amounts of JH. The abdominal store of JH is derived from the secretory activity of the corpora allata (CA) since removal of these glands prevented accumulation of JH in the abdomen (Williams, '56, '63; Schneiderman and Gilbert, '64). Even though in "pupal assays" both male and female CA demonstrated high activity, storage of JH is a unique feature of male abdomens (Williams, '59, '61). Recently, a careful analysis of the distribution of radiolabeled JH in abdominal tissues of moths injected with ³H-methionine led to the unexpected finding that the physiological basis of JH storage in male moths was due to the unusual characteristics of the accessory sex glands (ASG) (Shirk et al., '76). The ASG were found to contain a specific methyltransferase which methylates the JH acids to the respective hormones (Weirich and Culver, '79). A high proportion of the stored JH (Shirk et al., '76) as well as

the JH acid methyltransferase (JHAMT) (Weirich and Culver, '79) was found in the luminal material of the ASG. The JHAMT was also found to be present in the tissues of the ASG as well (Weirich and Culver, '79).

The accumulation of JH in the abdomen does not begin until just prior to (Gilbert and Schneiderman, '61) or coincident with (Metzler et al., '71) adult eclosion. By transplanting CA from pharate adults of various ages into pupae, Williams ('61) showed that the CA of both sexes beginning at about 15-16 days of adult development (almost a week prior to adult eclosion) can have a juvenilizing effect on the host pupa (which infers secretion of JH active material). In the ASG, high JHAMT activity was demonstrated even as early as day 14 of adult development (Weirich and Culver, '79). Thus, both components involved in JH accumulation attain functional competence long before adult eclo-

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sion, but the process of JH accumulation begins only at eclosion.

Although storage of JH is a unique feature of the adult male *Cecropia*, the CA of the two sexes also differ in the postmetamorphic period. There is no apparent sexual dimorphism with respect to either structure or activity during larval and pupal periods. During metamorphosis to the adult, the male CA undergo a marked increase in size and the large, lobulated adult male CA have a wet weight about five times more than female glands. Therefore, it was suggested that the much higher JH content in the male stems from the greater secretory capacity of the male CA (Gilbert and Schneiderman, '61). To test whether a sexual dimorphism in the secretory activity of CA indeed exists, the secretory products of CA maintained *in vitro* were monitored. In addition, we have employed the ability of the male ASG to sequester and store JH to provide an *in vivo* assay. Transplantations of male or female CA into decapitated newly eclosed moths or moths allatectomized as pupae, followed by injection of radiolabeled methionine, showed that abdomens with male CA contained far more radiolabeled JH than those with female CA (Dahm et al., '76). Is the dimorphic development of the CA due to differences between male and female internal milieu during metamorphosis? Is there a mutual interaction between the developing CA and ASG in males that leads to the characteristic adult pattern of activity in these two glands? In this paper, we describe and discuss the results of experiments carried out to answer these questions.

MATERIALS AND METHODS

H. cecropia were purchased from commercial suppliers as diapausing pupae and were kept at 4°C for at least 90 days. Adult development, which lasts 21–22 days, was initiated by exposure to 27°C, 70–80% relative humidity and 16:8-hr light-dark regime. The stage of development was determined by the criteria of Schneiderman and Williams ('54). All chemical reagents were reagent grade or better. The ether was anhydrous, analytical reagent grade (Mallinckrodt). Diazomethane was prepared from Diazald (Aldrich) in an alcohol-free ethereal solution according to the manufacturer's procedures. The [³H-7-ethyl]-JH-I acid was prepared from [³H-7-ethyl]-JH-I (14.1 Ci/mole) (New England Nuclear) by

saponification in these laboratories. The [³H-methyl]-methionine (8.8 Ci/mole) and [¹⁴C-methyl]-methionine (56 mCi/mole) were purchased from Amersham. The [³H-methyl]-methionine (3.7 Ci/mole) was purchased from Schwarz/Mann and the [1, 2-¹⁴C]-acetate (60 mCi/mole) from New England Nuclear.

Histology

The ASG were removed and immediately fixed in 3% glutaraldehyde and 3% acrolein in a 0.1 M cacodylate-0.05 M sucrose buffer (pH 7.4) and postfixed in 2% osmium tetroxide. The glands were embedded in Epon Araldite (Mollenhauer, '64) and 1- μ m sections were cut. The staining was a modification of the techniques described by Humphrey and Pittman ('74).

Injection of animals

After narcotization with CO₂, the moths were injected. All injected materials were dissolved in 25 μ l of Weevers' saline solution (Weevers, '66). For the *in vivo* labeling of JH, 2.8 nmoles (25 μ Ci) of [³H-methyl]-methionine was injected into each moth.

Allatectomy

Prior to initiation of adult development, a 2-mm hole was cut in the frontoclypeus, the epidermis was split longitudinally, and the corpora allata were removed by cutting the attached trachea. During all of the surgeries described, the animals were narcotized with CO₂, filled with Weevers' saline, treated with penicillin-streptomycin, and the wounds were sealed with wax.

Castration

The male pupae were castrated by cutting a slit dorsally in the fifth abdominal segment and removing the testes with forceps. The ovaries were implanted into the male pupae by cutting a slit in the fifth abdominal segment and slipping the ovaries into the hemocoel through the wound.

Extirpation and transplantation of the Herold organ

The Herold organ (Herold, 1815) is the imaginal disk for the ASG, seminal vesicles, common duct, and external genitalia of the adult male reproductive tract (Verson and Bisson, 1896). Removal of the Herold organ was done within 24 hr after initiation of adult

development by cutting a 3×3 mm hole in the integument of the ninth abdominal segment circumscribing the phallomeres of the pupa and clipping the organ free. To transplant the Herold organ, a 1-cm slit was cut dorsally in the integument of the fourth abdominal segment of the host. The Herold organ was picked up in Weevers' saline with a wide mouth transfer pipette which was inserted into the slit. The Herold organ was then expelled into the hemocoel so that the implant was not in contact with the epidermis.

Parabiosis

Parabiosis of the male and female was made in two orientations: Either the male head was attached to the female abdomen or the male abdomen was attached to the female abdomen. The head-abdomen parabiosis was created by cutting a 5-mm hole in the dorsal cuticle of the eighth abdominal segment of the female and then removing the underlying epidermis and similarly removing the cuticle and epidermis from the area of the frons of the male and uniting them with wax so that a clear passage between the two existed. In the abdomen-abdomen orientation, the tip of the abdomen was removed at the level of the tenth abdominal segment, making sure not to disturb the Herold organ of the male and again uniting the partners with wax so that a free passage existed. Extreme care was taken in all cases to eliminate any air bubbles by filling the animals with Weevers' saline.

In vitro incubations

The organs (ASG or CA) were removed from the animal and placed in Weevers' saline. All of the attached fat body and debris were removed. The ASG were cauterized proximally to seal the luminal contents inside. The organs were transferred to Grace's Insect Tissue Culture medium without methionine supplemented with 1% (w/v) bovine plasma albumin fraction V and 1% penicillin-streptomycin and then to a solution of Grace's without the antibiotics. The organs were finally transferred to the incubation medium and maintained at 25°C. Incubation Medium 1 consisted of Grace's Insect Tissue Culture Medium without methionine (GIBCO, Grand Island NY) supplemented with 1% (w/v) bovine plasma albumin fraction V and 36 nmole/ml [14 C-methyl]-methionine (56 mCi/mmole). In-

cubation Medium 2 was essentially the same except that 9 nmoles/ml [3 H-methyl]-methionine (3.7 Ci/mmole) was added as a methionine source

JH isolation

The JH was isolated from the ASG as previously described (Shirk et al., '76). In essence, the ASG were extracted with ether-ethanol (6:1), the extract was resolved by thin-layer chromatography (TLC) with Silica Gel HF₂₅₄ using a hexanes/ethyl acetate/acetic acid (70:25:5) solvent system and the JH zone eluate, as determined by cold side markers, was resolved by high-pressure liquid chromatography (HPLC) with a μ Porasil column using a hexanes/ethyl acetate (3.5%)/2-propanol (0.02%) solvent system. Scintillation counting was done in Liquifluor (New England Nuclear) with a Searle Mark III scintillation counter. The amount of JH-I acid present was determined by reacting the JH-I acid zone eluate from TLC with diazomethane two times and then measuring the radioactivity in the JH-I zone after resolution by HPLC.

RESULTS

The Accessory Sex Glands

The structure of male ASG. As previously described by Shepard ('74), the ASG were found to be paired organs which extend distally from and open into the seminal vesicles (for review see Leopold, '76). They ranged in length from 30 mm to 60 mm and had a thin layer of longitudinal and transverse muscles surrounding the secretory cells which lined the central lumen.

In the 16-day pharate adult, the gland cells were columnar with distinct boundaries. The large nuclei contained numerous granules showing a bluish stain. Similarly staining granules were also found in the narrow lumen close to cell boundaries (Fig. 1A). The presence of the granules in the lumen showed that the secretion of luminal material had begun at this stage. By day 19 the cell size had decreased and the granular material in the nuclei had disappeared. The enlarged lumen was filled with secretions which were tinged red-bluish violet. Small secretory granules were found toward the periphery, and in the central portion of the lumen the granules had coalesced to form large lumps (Fig. 1B). In the 21-day pharate adult, the

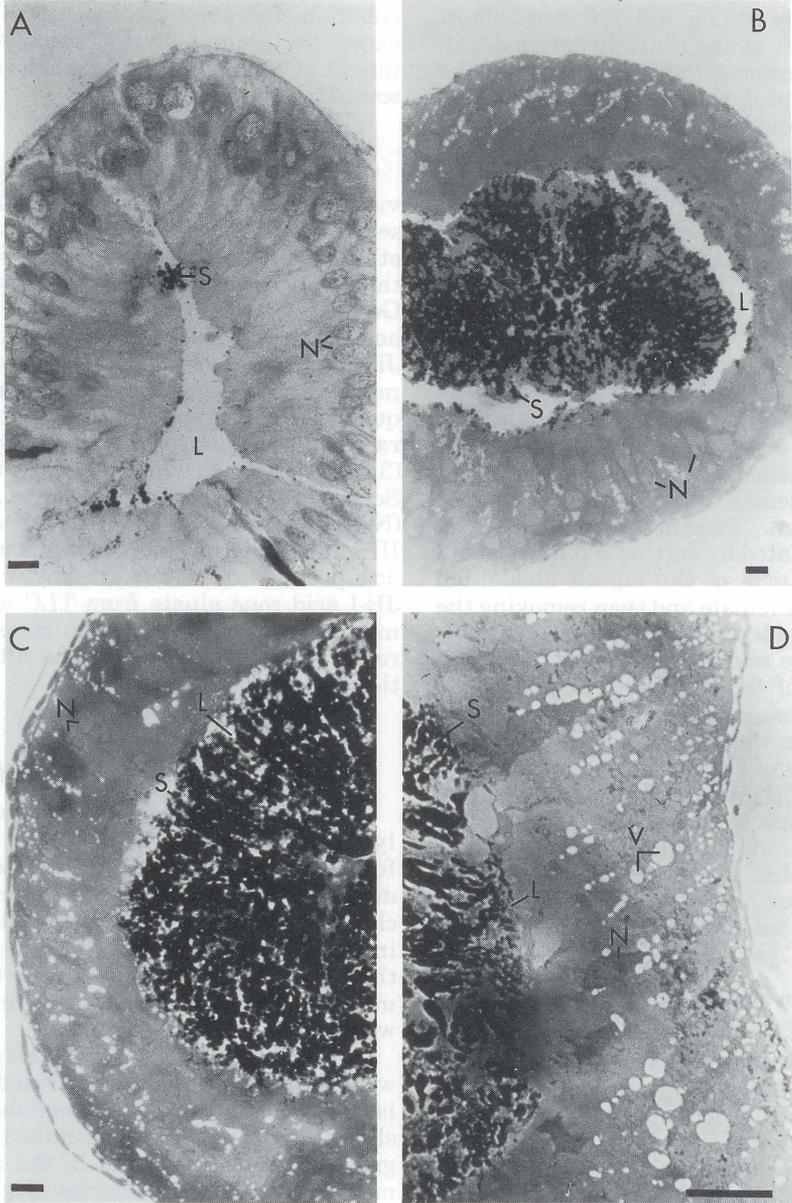


Fig. 1. Cross sections of ASG from: (A). 16-day pharate adult male *Cecropia* (the beginning of secretion can be seen by the presence of a few secretory granules in the lumen of the gland), (B). 19-day pharate adult male

Cecropia, (C). 21-day pharate adult male *Cecropia*, and (D). 5-day-old adult male *Cecropia*. G, secretory granules; L, gland lumen; N, nucleus; V, vacuoles. The bar equals 10 μm .

lumen was densely packed with large masses of secretory material (Fig. 1C). There was no further change in the cells or the stainability of the secretory material. Thus, the glands appeared to be functionally ready by the time

of eclosion of the moth (days 21–22). After 5 days, the males were becoming senescent, and the structures of the ASG were not excluded from this process. The gland cells of the ASG appeared to be degenerating; the

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cell boundaries were indistinct, most of the nuclei had disappeared and large vacuoles were present throughout the tissue (Fig. 1D).

Methylation of JH-I acid and accumulation of JH-I in ASG of pharate adults. Pharate adults staged to the tenth, 12th, 16th, 19th, and 21st day of development and newly eclosed adults were injected with 3.2 pmole [³H-7-ethyl]-JH-I acid. After 24 hr the animals were sacrificed, and the ASG were extracted and analyzed for JH-I and JH-I acid (Table 1).

Methylation of injected JH-I acid and accumulation of labeled JH-I were first detected on the 16th day of development. In one of the animals, the HPLC JH-I zone contained radiolabeled material which represented 0.26% of the total injected label. The yield of JH-I increased slightly on the 19th day and dramatically in one of the 21-day animals. The JH-I recovered from the two newly eclosed males represented 5% of the injected JH-I acid and agrees with the previ-

ously reported rate of conversion (Metzler et al., '72).

Free labeled JH-I acid was not detected in the ASG until the 19th day of development and even in adults the level never exceeded 0.25% of the total injected material. More importantly, even though the ASG were well formed by day 12 there was no indication for the uptake of injected JH-I acid by the glands.

Specificity of methylation of JH acids by the ASG. When challenged with the JH acids, the methyltransferases in the ASG esterify all three acids to their respective hormones. To test whether in vivo there is any preferential accumulation of the hormones, adult males allatectomized as pupae were injected with all three of the JH acids (10.0 µg each of JH-I acid, JH-II acid, and JH-III acid) along with [³H-methyl]-methionine. Analysis of the glands 2 days later showed the presence of radiolabel in all three JH zones, and the quantities were JH-II > JH-I > JH-III (Table 2). The quantities of JH reported are based on the incorporation of the methyl label without consideration of the dilution of radiolabeled methionine and therefore represent a minimum quantity of JH accumulation. Although these data demonstrate an inequality in the rate of JH accumulation in the ASG, it is possible that other factors such as the rate of breakdown of the three acids in the animal or specificity of hemolymph binding proteins may have caused the observed differences in the accumulation of the three hormones. Therefore, to eliminate some of the variables which occur in vivo the experiment was done in vitro. ASG, from adults allatectomized as pupae, were incubated for 24 hr in Medium 1 which contained a strictly quantified mixture of all three JH acids. Radiolabel was found coincident with only JH-I and JH-II zones (Table 2). Furthermore, unlike the in vivo experi-

TABLE 1. Conversion of JH-I acid and accumulation of JH-I in ASG of developing male cecropia

Age of adult males	Quantities accumulated in ASG	
	JH-I (fmoles)	JH-I acid ¹ (fmoles)
10-day pharate	nil ²	nil ³
12-day pharate	nil ²	nil ³
16-day pharate	nil ²	nil ³
	8.3	nil ³
19-day pharate	10.9	6.4
	22.7	1.0
21-day pharate	3.8	2.1
	179	8.0
Freshly eclosed	142	3.2
	168	1.6

¹Animals were injected with 3.2 pmoles of [³H-7-ethyl]-JH-I acid.

²Nil ≤ 2.4 fmoles, the smallest quantities detectable.

³Nil ≤ 1.8 fmoles, the smallest quantities detectable.

TABLE 2. Specificity of ASG for JH acids in vivo and in organ culture

Treatment	No. of trials	JH-I (pmoles)	JH-II (pmoles)	JH-III (pmoles)
In vivo ¹	2	27.2	47	7.1
In vitro incubation ²	6	299 (62) ³	22(12)	<0.1

¹Animals were injected with 10 µg each of JH acids and [³H-methyl]-methionine. The quantities of JH accumulated are based on the amount of radiolabel incorporated and therefore represent a minimum value.

²ASG were incubated in vitro with 10 µg each of the JH acids and [¹⁴C-methyl]-methionine. The quantities of JH accumulated are based on the amount of radiolabel incorporated and therefore represent a minimum value.

³Mean (standard deviation).

ments the yield of JH-I far exceeded that of JH-II.

Effect of internal milieu on the JH accumulation-related functional maturation of the ASG. Earlier, Gilbert and Schneiderman ('61) reported on the effects of parabiosis between male and female pupae on JH accumulation. After metamorphosis, only a quarter of the amount of JH normally found in a single male was accumulated in the adult male-female parabionts. A similar diminution in JH accumulation was observed when ovaries were transplanted into castrated male pupae. These data suggested the presence of a specific factor(s) in developing females which inhibits the development of the capacity of males to accumulate JH. Since the ASG constitute one of the major participants in the process of JH accumulation in males, we investigated the effects of a female environment on ASG development.

A series of cross transplantations was conducted to assess the effect of metamorphosis in a female on JH accumulation in ASG. First, Herold organs were implanted into allatectomized-Herold organectomized male pupae and allatectomized female pupae. When the adults eclosed, 100 μg of JH-I acid plus [^3H -methyl]-methionine were injected into the moths. The moths were sacrificed 24 hr later, and the ASG were removed and extracted for JH. ASG implants in females accumulated only 15% of the amount of JH in ASG implants in males (Table 3A, B) which was significantly lower ($P < 0.01$). These data show that culturing ASG during development in a female has the effect of decreasing the ASG capacity to accumulate JH-I from injected JH-I acid. However, the possibility remains that the observed effect was due to differences in the metabolism of the JH-I acid between the two sexes rather than to an alteration of ASG physiology. To test this possibility, ASG cultured in males or females were removed after adult eclosion and incubated *in vitro* with 100 μg JH-I acid and labeled methionine (Medium 2). At the end of 72 hr of incubation, the ASG were extracted and analyzed for labeled JH-I. ASG cultured in males had accumulated more than twice the amount of labeled JH obtained from ASG cultured in females (Table 3C, D). Although the JH accumulated in the ASG from female hosts was significant at a low level of confidence from males ($P < 0.1$), the observation is consistent with the inter-

pretation that either the female environment adversely affects the functional maturation of the ASG, or the male environment has a positive influence on the developing ASG.

That the female environment has an effect on the ability of the ASG to accumulate JH was also shown through indirect evidence. Allatectomized female pupae received implants of Herold organs, and after adult eclosion also received adult male CA transplants. When analyzed for JH labeled by [^3H -methyl]-methionine, ASG which had undergone metamorphosis in a female (Table 3I) accumulated only about 10% of the JH in ASG from similarly treated males (Table 3F, G). If ASG from female hosts were removed and transplanted into Herold organectomized males (Table 3H), the ASG accumulated less than 15% of the JH of the male controls (Table 3G). Implantation of pupal ovaries into male pupae had no effect on the ability of the ASG to accumulate JH (Table 3K). (The ovaries had developed normally in the male and there were approximately 200 oocytes in the male host.) Although these data suffer all of the problems of complex tissue and surgical interactions, the evidence suggests that development of the ASG in a female does cause a decrease in the capability to accumulate JH.

To make a more general assessment of the influence of females on male development, male pupae were parabiosed to female pupae either head to abdomen or abdomen to abdomen as described in the Materials and Methods. This was done to ensure that in at least one orientation there would be no possible damage to the Herold organ of the male which could cause a defect in the ability of the ASG to accumulate JH. After eclosion, the parabiosis was maintained and the male parabiont was injected with [^3H -methyl]-methionine. The JH accumulated in ASG from male parabionts in the head to abdomen orientation (Table 3L) was not different from normal males (Table 3E). However, the JH found in ASG of males in the abdomen to abdomen orientation was only 35% of the normal males (Table 3M). Although this appears to be a substantial decrease, it is not significantly different from the normal males. This may be due in part to the failure of two of the male parabionts to accumulate any radiolabeled JH. We can only speculate that even though the ASG appeared normal in size and secretions there may have been

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TABLE 3. Ability of ASG to accumulate JH after metamorphosis in a male or female host

Trial	Host for ASG metamorphosis	Implant into host pupa	Treatment after metamorphosis	No. of trials	JH (pmoles) accumulated in ASG			Total JH per pair CA
					JH-I	JH-II	JH-III	
A ¹	CA ⁻ /HO ⁻ male ²	HO	Injected with 100 µg JH-I acid	2	1.8			
B	CA ⁻ female	HO	Injected with 100 µg JH-I acid	3	0.27 (0.2) ³			
C	CA ⁻ /HO ⁻ male	HO	Removed ASG and incubated in vitro with 100 µg JH-I acid	2	1,200			
D	CA ⁻ female	HO	Removed ASG and incubated in vitro with 100 µg JH-I acid	3	610(260)			
E	Male	None	None	21	1.36 (0.75)	0.21 (0.14)	<0.026	1.57 (0.81)
F	CA ⁻ male	None	Adult male CA implanted	3	0.61 (0.49)	0.14 (0.063)	<0.004	0.75 (0.54)
G	CA ⁻ /HO ⁻ male	HO	Adult male CA implanted	3	0.39 (0.34)	0.29 (0.22)	<0.002	0.68 (0.54)
H	CA ⁻ female	HO	Removed ASG and transplanted to HO ⁻ adult male	6	0.09 (0.1)	<0.004	<0.004	0.1 (0.11)
I	CA ⁻ female	HO	Adult male CA implanted	4	0.028(0.018)	0.033(0.024)	<0.002	0.059(0.039)
J	CA ⁻ female	HO + pupal male CA	None	9	0.097(0.051)	0.056(0.051)	<0.002	0.12 (0.09)
K	Male	Ovaries	None	4	1.0 (0.05)	0.27 (0.17)	<0.015	1.27 (0.2)
L	Male/female parabiosis head to abdomen	None	None	4	1.15 (0.48)	0.24 (0.16)	<0.02	1.53 (0.62)
M	Male/female parabiosis abdomen to abdomen	None	None	8	0.47 (0.43)	0.08 (0.07)	<0.008	0.55 (0.44)

¹All animals were injected with 2.8 nmoles [³H-methyl]-methionine except for C and D which were incubated with 36 nmoles [¹⁴C-methyl]-methionine. The amount of JH accumulated is based on the amount of radiolabel incorporated and therefore represents a minimum value.

²CA⁻: allatectomized; HO⁻: Herold organectomized.

³Mean (standard deviation).

some surgery induced trauma which caused these observations.

The release of JH from the male ASG. Williams ('61) parabiosed adult male abdomens with chilled pupae and observed massive juvenilization of the developing partner. Since we know that the JH is almost entirely stored in the ASG, it seemed desirable to repeat these experiments with special care to prevent damage to the ASG in order to confirm that the stored JH is indeed released by the ASG into the hemolymph. Abdomens of normal and allatectomized (as pupae) adult males were parabiosed with normal and allatectomized female pupae. At the end of the adult development the animals were scored for the degree of juvenilization incurred by this treatment. All surviving partners of the allatectomized adult male abdomens developed into normal adults (four of 12 survived). The females parabiosed to normal male abdomens showed extensive juvenilization with all animals molting to second pupae (five of ten survived). Since some of the female pupal partners lacked their own CA, the juvenilization could only have been caused by the release of JH from the ASG of the adult male partner, which agrees with the previous observations of Williams ('61).

The corpora allata

Our previous studies clearly established the significance of the role of the ASG in accumulation of JH in male *Cecropia* moths.

However, the unique capacity of the ASG to methylate and store JH may not be the only factor in the sexual dimorphism of JH accumulation. The CA of adult males are much larger than those of females (Gilbert and Schneiderman, '61) and correspondingly appear to secrete greater quantities of JH-related compounds than female CA (Dahm et al., '76; Peter et al., '81). Is the sexual dimorphism in CA due to an intrinsic program of metamorphosis or is it due to factors in the developing internal environment? To answer this question, cross-specific transplantation of CA between male and female pupae were carried out, and the secretory activity of these CA was evaluated using the accumulation of JH in the ASG as an index of the biosynthetic activity after metamorphosis.

The biosynthetic activity of adult male CA in vivo. The biosynthetic capacity of transplanted male and female CA was evaluated in adult males allatectomized as pupae. CA from newly eclosed males and females were transplanted and [³H-methyl]-methionine was injected into the allatectomized hosts. Two days later the ASG were removed, and the radiolabeled JH was quantitated. The controls consisted of male moths with intact CA. The data (Table 4) show that on a per pair basis the JH content of hosts with transplant male CA did not differ significantly from those with CA in situ. On the other hand, the ASG of moths with transplanted female CA contained significantly less radiolabeled JH.

TABLE 4. JH produced by male and female CA cultured *in vivo* and accumulated in ASG¹

Treatment	No. of trials	Accumulation of JH (fmole/pair CA) in ASG ¹			
		JH-I	JH-II	JH-III	Total JH per pair CA
Normal males	21	1,360 (750) ²	210 (140)	<26	1,570 (810)
CA ⁻ Male plus adult male CA	3	600 (480)	140 (60)	<4	750 (540)
CA ⁻ male plus adult female CA ⁴		16	56	ND ⁵	72
Male CA metamorphosed in CA ⁻ male	~	580 (360)	400 (260)	<5	950 (580)
Female CA metamorphosed in CA ⁻ male	3	4 (6)	12 (6)	<5	16 (11)
Male CA metamorphosed in CA ⁻ female transplanted into CA ⁻ male	5	900 (645)	380 (225)	<9	1,230 (760)

¹Animals were injected with [³H-methyl]-methionine. Quantities of JH present computed on the basis of total dpm incorporated into JH and therefore represent a minimum value.

²Mean (standard deviation).

³CA⁻: allatectomized.

⁴Data from Dahm et al. ('76).

⁵ND: Not determined.

Next, we transplanted male pupal CA into female pupae and vice versa to test whether the difference in CA activity was due to factors in the internal environment during the metamorphic period. Three types of transplantation were carried out: (1) male pupal CA into allatectomized male pupae, (2) female pupal CA into allatectomized male pupae, and (3) male pupal CA into allatectomized female pupae. After adult eclosion, the male CA from group 3 females were re-transplanted into newly eclosed allatectomized male moths. All these adults were injected with [^3H -methyl]-methionine, and 2 days later the ASG were extracted. The ASG of animals with male CA transplanted in the pupal stage (Group 1) and with male CA which developed in female pupae (Group 3) contained radiolabeled JH in amounts which were not significantly different from that of ASG from moths with intact CA. The ASG of moths which received female CA transplants in the pupal stage (Group 2) had much less JH than both the above groups (Table 4). From these data, we can conclude that the sexually dimorphic development of the CA production of JH related compounds is not dependent upon the environment of the metamorphosing CA.

Biosynthetic activity of larval and adult CA incubated in vitro. Earlier findings from this laboratory have demonstrated the lack of the JHAMT activity in the CA of adult male *Cecropia* which is necessary to esterify the JH acids prior to secretion in vitro (Peter et al., '81; Dahm et al., '81). Considering the important role of JH during metamorphosis, we found it critical to determine whether the CA of male larvae also lacked the JHAMT activity or whether the lack of JHAMT in adult males represented a secondary loss of the enzyme during the metamorphic period. To test for the presence of the JHAMT, we identified the secretory products of CA incubated in vitro. CA from early male and female fifth instar larvae (<24-hr-old) were incubated in Medium 1 for 4 days. The medium was then extracted and analyzed for JH related compounds. The CA from males produced both JH-I (0.15 fmole day $^{-1}$ pair $^{-1}$) and JH-II (0.41 fmole day $^{-1}$ pair $^{-1}$) but not JH-III (<0.08 fmole day $^{-1}$ pair $^{-1}$). Female CA produced similar amounts: 0.16 fmole day $^{-1}$ pair $^{-1}$ JH-I and 0.95 fmole day $^{-1}$ pair $^{-1}$ JH-II. The presence of the bona fide JHs in the incubation media suggests that the JHAMT is present in the CA of both

male and female larvae. Adult male CA were incubated in Medium 2, which also contained [1, 2- ^{14}C] acetate. The use of this medium made it possible to detect the presence of JHAMT activity as well as obtain a relative measure of the quantities of sesquiterpene derivatives being produced by the CA. However, since the medium was not made deficient in all carbon sources for the synthesis of sesquiterpenes, the acetate labeling would not allow for strict quantitation of the CA activity. The medium from freshly eclosed adult male CA contained 1,300 (\pm 1,600) dpm day $^{-1}$ pair $^{-1}$ JH-I acid and 1,200 (\pm 2,200) dpm day $^{-1}$ pair $^{-1}$ JH-II acid. No JH-III acid was detected even though the precursor farnesoic acid (1,650 (\pm 800) dpm day $^{-1}$ pair $^{-1}$) had been synthesized. Neither was there any evidence of the methylation of the JH acids. From these data, we have found that the larval CA of both sexes can synthesize and secrete the JHs, but that after metamorphosis the adult male CA have lost the ability to synthesize the complete JHs and secrete the JH acids instead.

DISCUSSION

Since active methylation of JH acids and storage of JH does not occur until adult eclosion (Table 1), the process of JH accumulation in the ASG appears to be developmentally regulated. Even though JHAMT activity is present in the tissues and luminal contents of ASG from 16-day pharate adults (Weirich and Culver, '79), accumulation of JH only begins shortly before or after eclosion. This may be due in part to the timing of initiation of secretory function by CA (Gilbert and Schneiderman, '61; Williams, '61) but equally important is the capacity of the ASG to take up JH acid. Our data show that the ASG become competent to accumulate JH-I on day 16 of adult development (Table 1), which is coincident with the onset of secretion of luminal material. However, the ability to accumulate JH-I from exogenous JH-I acid remains low until eclosion even though most of the luminal material, of which the JHAMT is one component, is secreted by day 19. It is only shortly before adult eclosion that the capacity to accumulate JH reaches adult levels. In agreement with this, we showed that the uptake of injected JH-I acid was low in ASG of pharate adults until just prior to eclosion. If the JH acid diffuses freely into the ASG, then it should have been present in the ASG of all ages of pharate adults

injected with JH-I acid. The regulation of JH accumulation may thus be at the level of JH acid transport through the gland cells.

The specificity of JH accumulation also reflects the parallel biochemical specialization of the CA and the ASG. The CA of adult *Cecropia* secrete only JH-I acid and JH-II acid in vitro (Dahm et al., '81; Peter et al., '81), and the ASG accumulate more JH-I than JH-II and virtually no JH-III when incubated with equal quantities of JH acids in vitro (Table 2). The JHMT in homogenates of ASG exhibit a similar substrate preference pattern (Dahm et al., '81; Peter et al., '81). However, a more complex picture emerges from the in vivo data. When equal quantities of the three JH acids were injected into all-tectomized adult males, the ratio of juvenile hormones accumulated in the ASG was different from that obtained by incubation of the acids with ASG in vitro. JH-II was the major component accumulated, but in contrast to the ASG in vitro, in the animals the glands accumulated substantially lower quantities of JH-I. This suggests that the uptake of JH acids may also be regulated by the transport of the acids to the glands and/or the rate at which the acids are cleared from the hemolymph. Lastly, the accumulation of JH in the ASG may also be influenced by the catabolic enzymes in the ASG itself; both JH esterase and epoxyhydrase are present in the ASG (Peter, Dahm and Röller, unpublished) and their substrate preference could affect the final ratio and quantities of JH accumulated.

The development of the ASG is affected during metamorphosis by the environment they are in. ASG which have metamorphosed in female pupae have a decreased capacity of JH accumulation. That this is not due to simple physical factors, such as postoperative trauma, is shown by the data from various cross-transplantation experiments. However, neither ablation of the brain-CC-CA complex (data not shown) nor the gonads had any influence on ASG development either positive or negative. Whether some male factor is necessary for stimulation or functional maturity of the ASG or a female factor suppresses is still uncertain. The fact that parabiosis with a female had no adverse effect on the ASG may indicate a role for a positive male factor, although dilution of a female suppressing factor cannot be excluded in these experiments.

The adult CA exhibit sexual dimorphism which is manifested by a difference in size, the nature of the secretory product (i.e., JH acids vs. JH), and the quantity of the product

secreted. On the basis of wet weight, Gilbert and Schneiderman ('61) determined the CA of adult males to be five times larger than CA of adult females. It was also found that the amount of JH acids in the adult male CA was substantially more than the amounts in female CA (Peter et al., '81). If one accepts that the amount of JH accumulated in the ASG is related to the quantity of product secreted from the CA, the large male CA were far more active than the female CA. Therefore, at least for this moth the size of the CA truly reflect the secretory activity. A correlation between productivity and CA size has also been demonstrated for the cockroach *Diploptera punctata* (Szibbo and Tobe, '82). As an antithesis to *Cecropia*, the males of this cockroach have small CA which produce JH at slower rates than do CA from females. In both of these animals, the evidence suggests that the capacity to synthesize JH is an intrinsic component of the CA.

The reasons underlying the hypertrophy of the male CA in *Cecropia* remain to be elucidated. We can, however, infer that differences in internal milieu during adult metamorphosis are not responsible for the dimorphism since male CA developing in females showed no decline in the amounts of JH-related compounds produced. On the other hand, neither did the female CA gain in their productivity by metamorphosing in the male environment. The determinative event for the dimorphism may therefore be genetically programmed or programmed at an earlier stage.

A final puzzling feature of the male JH system is the apparent loss of the JHMT activity in the CA during metamorphosis. This modification in the morphogenesis of the CA may be a fortuitous natural phenomenon, the study of which may provide clues to unravel the control mechanisms regulating CA activity in general. Current evidence suggests that the controls may be either neurohormonal, neural, or both. The neurohormones may be stimulatory (Sehnal and Granger, '75; Bhaskaran, '81) or inhibitory (Girardie, '67; Morohoshi and Shimada, '76; Williams, '76; Bhaskaran et al., '80; Bhaskaran, '81). The neural control is predominantly inhibitory (Wigglesworth, '36; Scharrer, '52; Lüscher and Englemann, '55; Fraser and Pipa, '77; Stay and Tobe, '81). Generally during the penultimate or last larval instar, the CA are inactivated or have greatly suppressed activity and then during pharate adult stage or after eclosion the CA become reactivated in almost all examples. The male *Cecropia* CA may represent a rare

instance of a failure to reactivate the expression of an enzyme involved in the terminal step of the biosynthetic scheme of JH.

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