The Accessory Sex Glands as the Repository for Juvenile Hormone in Male Cecropia Moths

Paul D. Shirk *, Karl H. Dahm, and Herbert Röller

Institute of Developmental Biology, Texas A&M University

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Gas chromatographic determinations, bioassays, and radiolabelling experiments show that the juvenile hormone in adult male *Hyalophora cecropia* is accumulated exclusively in the accessory sex glands. Moths do not store measurable quantities of juvenile hormone if their accessory sex glands are removed shortly after adult eclosion.

Adult males of some saturniid moths, in particular *Hyalophora cecropia* (L.), are unique in their ability to accumulate and store large amounts of juvenile hormone (JH)[1,2]. Without convincing evidence[3], it has been generally assumed that the hormone is sequestered in the fat body. During our studies on the biosynthesis of JH in adult male *H. cecropia*, we observed that the incorporation of the S-methyl group of methionine[4] is not necessarily controlled by corpora allata. Even after removal of these glands (allatectomy), male cecropia moth are able to replace the methyl ester group of juvenile hormones with the S-methyl group of radiolabelled methionine. Substrates for this reaction may be endogenous JH-I and JH-II biosynthesized prior to allatectomy, or exogeneous JH-I, JH-II and JH-III injected simultaneously with the methionine (unpublished results). This finding prompted us to search for the tissues responsible for the unusual accumulation of juvenile hormones. We quickly discovered that in *H. cecropia* JH-I and JH-II are sequestered exclusively in the male accessory sex glands. Four representative experiments may illustrate our results.

1. Two 48 h-old adult male *H. cecropia* were each injected with 25 μCi [S-methyl-^{3}H]-methionine. After a 48 h incubation period, the reproductive tract (moth 1) or the accessory sex glands plus seminal vesicles (moth 2) were dissected out. The reproductive organs were extracted separate of the remains. Aliquots of the extracts were tested for JH activity by the Galleria wax test[5]. The remaining extracts were processed for JH analysis finally by GLC ([6] and literature cited therein). Unlabelled juvenile hormones were added to the extracts of the carcasses in order to facilitate isolation of the biosynthetically labelled hormones. Juvenile hormones, as identified by radiolabel or by GLC, were found only in the fractions containing the accessory sex glands (Table I). The biological activities of these fractions were at least three orders of magnitude higher than those of the remainder of the carcasses.

2. A 24 h-old adult male *H. cecropia* was injected with 25 μCi [S-methyl-^{3}H]-methionine. After a 24 h incubation period, the reproductive tract was dissected from the moth and divided into its component organs: the accessory sex glands (clipped off at the junction with the seminal vesicles), the common duct (clipped proximally at the juncture with the seminal vesicles and distally at the juncture with the ejaculatory duct), the seminal vesicles, the testes, and the vasa deferentia (clipped proximally at the testes and distally at the constriction before the seminal vesicles). JH-I (21,000 dpm) and JH-II (4,100 dpm) were found only in the accessory sex glands. Analysis by TLC, high pressure liquid chromatography, and liquid scintillation counting showed that the other parts of the reproductive tract contained no radiolabelled JH.

3. The accessory sex glands were removed from five 96 h-old adult male *H. cecropia* and extracted. JH-I and JH-II were isolated and analyzed by GLC. The extract contained 12.8 μg JH-I (2.6 μg/moth) and 1.1 μg JH-II (0.2 μg/moth).

Table I. JH in different tissues of adult male cecropia. *nil*: not detectable, less than 35 dm. * Total JH activity in *Galleria* Units.

Moth	Fraction	JH Activity [GU *]	JH-I [dpm]	JH-I [μg]	JH-II [dpm]	JH-II [μg]
1	reproductive tract	20×10^6	32,000	1.4	12,000	0.4
1	remains of carcass	$<1 \times 10^3$	*nil*	—	*nil*	—
2	accessory sex glands plus seminal vesicles	5×10^6	6,200	2.1	590	0.3
2	remains of carcass	1×10^3	*nil*	—	*nil*	—

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Requests for reprints should be sent to Dr. H. Röller, Institute of Developmental Biology, Texas A&M University, College Station, *Texas 77843*, U.S.A.

4. The accessory sex glands were removed from two 1 hour-old adult male *H. cecropia*. Each animal was injected with 25 μCi [S-methyl-^{3}H]-methionine 24 h post-eclosion. After a 24 h incubation period, the moths were sacrificed. No radiolabelled JH was detected in extracts of the whole carcasses.

In lieu of direct evidence, it is reasonable to assume that the high JH activity in males of some other saturniid species is also associated with the accessory sex glands. We have been unable to isolate juvenile hormone from female moths. The sexual dimorphism with regard to JH may be attributed largely to the properties of the male accessory sex glands. The physiological significance of the JH accumulation remains unknown. It may represent a physiological vestige of former reproductive functions.

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