Those enteroglucagons show glucagon-like immunoreactivity only to "nonspecific" antibodies, which bind a part of the amino terminal of glucagon. Although some gut-type glucagons apparently contain the complete glucagon amino acid sequence, the antigenic site at amino acids 24 through 29 is not exposed [J. M. Conlon, in *Glucagon: Physiology, Pathophysiology, and Morphology of the Pancreatic A Cells*, R. H. Unger and L. Orci, Eds. (Elsevier, New York, 1981), p. 55; J. J. Holst, *Digestion* **17**, 168 (1978); J. B. Jaspan, K. S. Polonsky, A. H. Rubenstein, in *Glucagon: Physiology, Pathophysiology, and Morphology of the Pancreatic A Cells*, R. H. Unger and L. Orci, Eds. (Elsevier, New York, 1981), p. 77; A. J. Moody, M. Jacobson, F. Sundby, in *Gut Hormones*, S. R. Bloom, Ed. (Churchill Livingstone, Edinburgh, 1978), p. 369].
15. Radioassay Systems Laboratories gives the cross-reactivity of this antibody (as a percentage of its reactivity to pancreatic glucagon) as 0.0014 to gut-type glucagon, 0.0005 to porcine insulin, and 0.0003 to porcine or synthetic human gastrin.
16. Resting was defined as a stationary posture, with the abdomen supported by the cage floor and with no other behaviors displayed.
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Larval Testes of the Tobacco Budworm: A New Source of Insect Ecdysteroids

Abstract. *Testes of last-instar larvae of the tobacco budworm release five times more ecdysteroid into incubation medium (judged by radioimmunoassay) in 2.5 hours than is found in testis homogenates. Incubation of testicular components indicates that the testis sheath may be the site of ecdysteroid synthesis. Fractionation of hemolymph, testis homogenate, and incubation medium by high-performance liquid chromatography produces a distinct ecdysteroid pattern in each case. Thus, released testis ecdysteroids are probably converted to other forms for use, sequestration, or general circulation. Their functions are unknown.*

Ecdysteroids, particularly ecdysone and 20-hydroxyecdysone, are currently accepted as the molt and development promoting hormones in insects (*1*). The sources of ecdysone in immature insects are reported to be the prothoracic glands and, to a limited extent, oenocytes associated with epidermis or fat body (*2*). Anomalies, in which ecdysteroid-dependent development occurs in segments experimentally separated from the prothoracic glands, have been reported in at least four insect orders (*3*) and have prompted a search for other organs capable of considerable ecdysteroid synthesis. By the time insects reach adulthood the prothoracic glands degenerate (*1*), but ovaries of some adult females then become capable of secreting ecdysteroids into the circulating hemolymph (*4–8*). Even though testes of adult males contain ecdysteroids, their blood generally holds little or no detectable hormone (*9*). We report now that there is extensive ecdysteroid synthesis and release by isolated testes of late last-instar larvae of the tobacco budworm, *Heliothis virescens*.

Testes of laboratory reared *H. virescens* (*10*) were excised from last-instar larvae just before the pupal molt, at or just before fusion of the paired testes, and washed in sterile insect Ringer solution (*11*) with garamycin (250 mg/ml) (Schering). The osmolality of the Ringer solution was approximately the same as hemolymph of animals at this stage in development (285 mOsmole/kg) (*12*). Testes were separated into components by pulling them apart with forceps in medium and agitating them to expel germinal cells (spermatocysts) and follicle fluid from the sheaths. Sheath and the spermatocyst fractions were rewashed prior to incubation in the Ringer medium. Follicle wash-fluid was saved for analysis by radioimmunoassay (RIA) (*13, 14*). At the end of the incubation period, all tissues and medium samples were processed for RIA. Other tissues (fat body, jaw muscle, hemolymph) were taken simultaneously from the testis donors; brains were taken from 4th-instar larvae. Ecdysteroids were secreted into the medium by intact testes and testis sheaths (Fig. 1) but not by spermatocysts (with or without follicular fluid), muscle, fat body, or brain (Table 1).

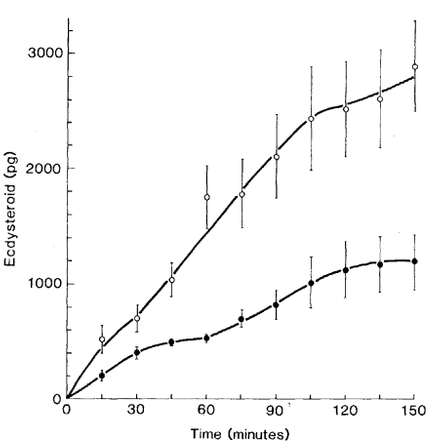

Fig. 1. Ecdysteroid in medium surrounding incubating pairs of whole testes (open circles) and testis sheath-pair equivalents (closed circles). Ecdysteroid content of 100-μl samples, withdrawn every 15 minutes, was determined by RIA (*13*). Fused and nonfused testes were pooled before the experiment; two nonfused testes or one fused testis, incubated in 200 μl of medium, were considered a testis pair. The ordinate represents cumulative data at each time interval. Error bars represent standard errors determined for each point in six separate experiments.

Intact testes released approximately twice as much RIA-detectable ecdysteroid as sheath preparations during the incubation period. More than half of the RIA-sensitive ecdysteroid of whole testes ($N = 26$) was found in testis follicular fluid (means ± S.D.: 53 ± 7 percent); 20 ± 8 percent was in sheath preparations, and 8 ± 2 percent appeared in washed spermatocysts. The ecdysteroid synthesis tissue appears to be part of the sheath of the testis; it may therefore be analogous to the ecdysteroid-synthesizing follicular epithelium of adult female insect ovaries (*6–8*). High-performance liquid chromatography (HPLC) fractionation (*15*) of methanol extracts of hemolymph, whole testes, and whole testis incubation medium (2.5 hours) followed by RIA of resulting fractions, elicited the ecdysteroid patterns shown in Fig. 2. Peaks E, G, H, and I were common to all preparations. Peaks E and G cochromatographed in the same solvent system with makisterone A and 20-hydroxyecdysone, respectively. Peaks H and I probably correspond to 20,26-dihydroxyecdysone and a highly polar component, respectively (*16, 17*). Hemolymph and whole testes also showed peak C (cochromatographed with ecdysone in our system), peak F_1 (cochromatographed with 26-hydroxyecdysone), and peak A (unidentified). Hemolymph and testis ecdysteroid patterns are similar to those described at various times in the life cycles of other insects and as metabolites of ecdysone (*7, 16–18*).

Although the antibody used for RIA in this study is highly sensitive to ecdysteroids, it does not bind each analog to the same degree (*14*) so that the height of each ecdysteroid analog peak in Fig. 2 may not reflect the actual amount of analog. However, the relative titers of corresponding peaks may be compared. Peak F_1 is about twice as high in the testis extract as in the hemolymph, while peak G is about half as high. Therefore the proportion of hormones in testis tis-

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Table 1. Ecdysteroid production (analysis by RIA, in 20-hydroxyecdysone equivalents) by incubated tissue of *Heliothis virescens*.

Tissue	Ecdysteroid in each	Ecdysteroid in non-incubated control		Ecdysteroid after 2.5-hour incubation		
		N	Tissue* (pg)	*N*	Tissue* (pg)	Medium (pg)
Whole testis	Testis pair	7	796 ± 15	6	861 ± 218	2898 ± 597
Testis sheaths	Testis pair	3	424 ± 214	6	385 ± 73	1190 ± 239
Spermatocysts in follicle fluid	Testis pair	2	385 ± 70	3	469 ± 58	NS‡
Washed spermatocysts	Testis pair	3	66 ± 5	3	64 ± 16	NS‡
Fat body	1 mg†	1	199	2	50 ± 12	110 ± 19
Jaw muscle	1 mg†	1	26	2	7 ± 2	15 ± 9
4th-instar brains	Brain	2	70 ± 8	2	109 ± 67	0
Hemolymph						
20° short day	1 μl	5	685 ± 98			
30° long day	1 μl	6	3410 ± 408			
Gentomycin-Ringer	200 μl	7	0	7		0
Gentomycin-Ringer + 1000 pg of 20-hydroxyecdysone	200 μl			4		1074 ± 5

*± Standard error of the mean. †Wet weight. ‡NS, not separated.

sue may be different from that circulating in the blood. The ecdysteroid pattern of the testis incubation medium in the region less polar than peak G is different from the other tissue spectra; peak C is absent, and peaks F_2, L, M, N, O, P, and Q are detected. Ecdysteroids synthesized by insect ovaries are reported to be ecdysone (*4, 6, 7*), 20-hydroxyecdysone (*7*), or 2-deoxyecdysone (*5, 6, 8*). Prothoracic glands in vitro produce only ecdysone (*1, 18*); this is converted in vivo to other ecdysteroids by tissues such as fat body, epidermis, and Malpighian tubules (*7, 16–18*). The presence of peaks F_2 and L to Q and the absence of peak C in the testis medium ecdysteroid pattern indicates that *H. virescens* testes are releasing a characteristic set of steroids, which are probably converted to the forms found in whole testes and hemolymph or utilized by tissues not present in our incubation system.

As in *Locusta* ovaries (*7*), about 17 percent of the RIA-detectable testis ecdysteroids appear as a highly polar fraction (peak I in Fig. 2). However, the entire polar peak of the *Locusta* ovary extract can be hydrolyzed with snail glucosylase-sulfatase (Sigma) to yield approximately 50 times more RIA-detectable nonconjugated ecdysteroids than detected in the untreated polar peak (*7*). In contrast, only 51 ± 5 percent (approximately 12 testes per analysis, $N = 4$) of the *H. virescens* testis polar peak fraction was hydrolyzable by snail glucosylase-sulfatase. HPLC and subsequent RIA of the enzyme-treated polar peak yielded ecdysteroid-positive fractions whose sum (in picograms of 20-hydroxyecdysone equivalents) was approximately the same as the difference between the nonreacted and reacted polar component. Therefore, conjugated ecdysteroids do not appear to be a considerable storage reserve for ecdysteroids in *H. virescens* testis tissue at this stage, nor are they the primary source of ecdysteroids liberated into the medium during in vitro incubation.

It seems unlikely that an actively secreting pair of testes produces enough ecdysteroid to maintain pre-molt hemolymph concentrations, particularly at the rapid turnover rates prevalent at times of high ecdysteroid titer (*17*). Therefore, prothoracic glands, and possibly oenocytes, are probably secreting most of the ecdysteroids circulating in *H. virescens* blood at the time the testes are also actively secreting. In contrast, adult locust ovaries are thought responsible for secretion of all circulating ecdysteroids at that time (*6, 7*). Ovary ecdysteroids reportedly stimulate egg yolk production and are stored in the eggs for later use by developing embryos (*6, 7, 16*). What, then, could be the function of testis ecdysteroids secreted prior to the pupal molt? Testes may be safety devices, assuring additional sources of molting hormone. Alternatively, testis ecdysteroids may specifically stimulate mitotic divisions during early stages of spermatogenesis (*19*), interact with hemolymph macromolecular factor (*20*) to induce further spermatocyst differentiation, or influence the development of apyrene sperm (*21*) or the male reproductive tract (*22*) (or both) which occur later.

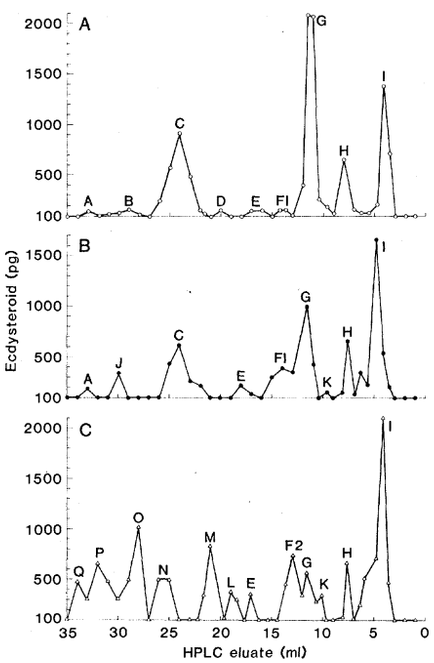

Fig. 2. Ecdysteroid patterns generated by RIA of HPLC fractions (*15*) of 75 percent methanol extracts of hemolymph (A), whole testes (B), and incubation medium after exposure for 2.5 hours to whole testes (C). All analyses were done at least twice, and the patterns presented are composites of the data so obtained. (A) Data corresponding to 3 μl of hemolymph; (B) and (C) normalization to data from six testis pairs. Actually, 5-μl samples of hemolymph from 15 premolt male larvae were pooled and analyzed to produce (A); approximately 50 testes were extracted to produce (B); incubated medium from 12 testes pairs was extracted to produce (C).

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Photoreceptor Membrane Shedding and Assembly Can Be Initiated Locally Within an Insect Retina

Abstract. *Photoreceptors of locust compound eyes add new receptor membrane at dusk and shed membrane at dawn. When part of an eye is masked before dusk, premature assembly of new membrane is initiated in the masked ommatidia but not in the adjacent unmasked ommatidia. Similarly, masking some ommatidia just before dawn prevents normal shedding only in the masked ommatidia. Therefore, the shedding and assembly phases of photoreceptor membrane turnover can be initiated by a change in the state of illumination of individual ommatidia.*

The photoreceptor membrane (PRM) in vertebrates (*1*) and arthropods (*2*) turns over according to a daily cycle. During turnover, the amount of shedding of the rod outer segments in vertebrates and the rhabdomeres in arthropods normally reaches a peak after dawn, under the influence of the onset of light and of endogenous factors (*1–5*). Assembly of new rod outer segment disk is also greatest during the morning (*6*). However, in some arthropods, assembly of new microvillar PRM is restricted to a few hours just after dusk and, like shedding, appears to be controlled both endogenously and by a change in the ambient lighting (*2, 5, 7*).

Attempts to localize the control mechanisms of PRM turnover have shown that in various animals both shedding and assembly are controlled separately in each eye. Shedding in the frog *Rana* is prevented in one eye if that eye is masked at dawn, while shedding in the unmasked eye is not affected (*8*). When one eye of a crab is masked in the afternoon, new PRM is soon assembled in that eye but not in the other (*7*). Initiation of shedding, as well as synchronization of its circadian timing by the central nervous system, occurs unilaterally in rat eyes (*9*). Similarly, *Limulus* lateral eyes are affected independently by efferent input that is necessary for normal turnover (*4*). Isolation of single eyes in vitro does not prevent light-stimulated shedding of PRM in *Xenopus* eyes (*10*), or assembly of PRM in a locust (*11*) or crab eyes (*12*).

In the present experiments, I have attempted to localize the control of PRM shedding and assembly to within discrete regions of single compound eyes of a locust. Whereas all the photoreceptors of a vertebrate eye share one lens system, the compound eyes of insects such as locusts are composed of many optically isolated units known as ommatidia. I have taken advantage of this arrangement by using simple masking experiments to ascertain if shedding or assembly can be initiated locally in the retina without affecting adjacent regions.

Each locust ommatidium has eight receptor cells that contribute to a rhabdom of photoreceptive microvilli and share the same visual field (*13*). As in many arthropods, locust rhabdoms vary in size as a result of daily turnover (*5, 14*). Shedding by pinocytosis from the bases of the microvilli reduces their cross-sectional area four- to fivefold at dawn, while at dusk, assembly of newly synthesized PRM increases it by a corresponding amount (*5*); the night rhabdom is larger than the day rhabdom mainly because its microvilli are twice as long (*5, 14*). A change in rhabdom cross-sectional area is therefore a convenient and clear indication of the occurrence of PRM shedding or assembly.

When locusts are placed in darkness 5 hours before the normal time of dusk, premature initiation of the assembly of new PRM increases rhabdom cross-sec-

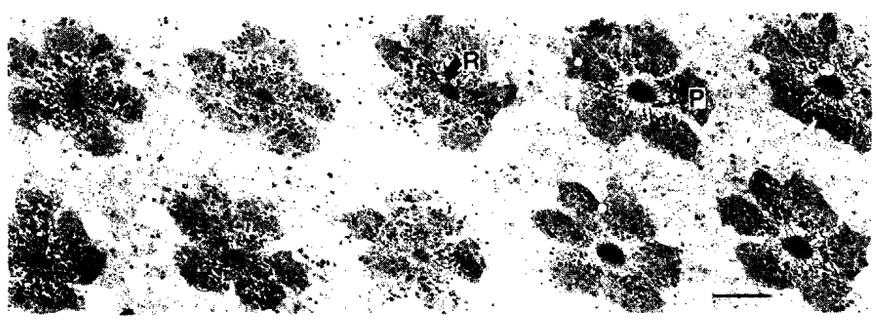

Fig. 1. Electron micrograph of a transverse section of ommatidia across the boundary between masked (right) and unmasked (left) parts of an eye. The ventral half of the eye was masked with tape 5 hours before dusk, and the eye was fixed 4 hours later. Over the central region of a locust compound eye, rhabdom size is normally the same among different ommatidia. Masking, however, has induced the four rhabdoms on the right to enlarge (they average 13 μm^2 cross-sectional area) by assembling new microvillar membrane. The four rhabdoms on the left have remained in the day state (average cross-sectional area is 5.3 μm^2). The two central rhabdoms may be in an intermediate state (6.5 μm^2) (*19*). The masked rhabdoms are surrounded by a "palisade" (*P*) of endoplasmic reticular vacuoles. Scale bar, 10 μm. *R*, rhabdom.

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