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MIDGUT OF LEPIDOPTERAN PUPAE IS A MAJOR DEPOT OF SEQUESTERED, MOBILIZABLE, ECDYSTEROIDS

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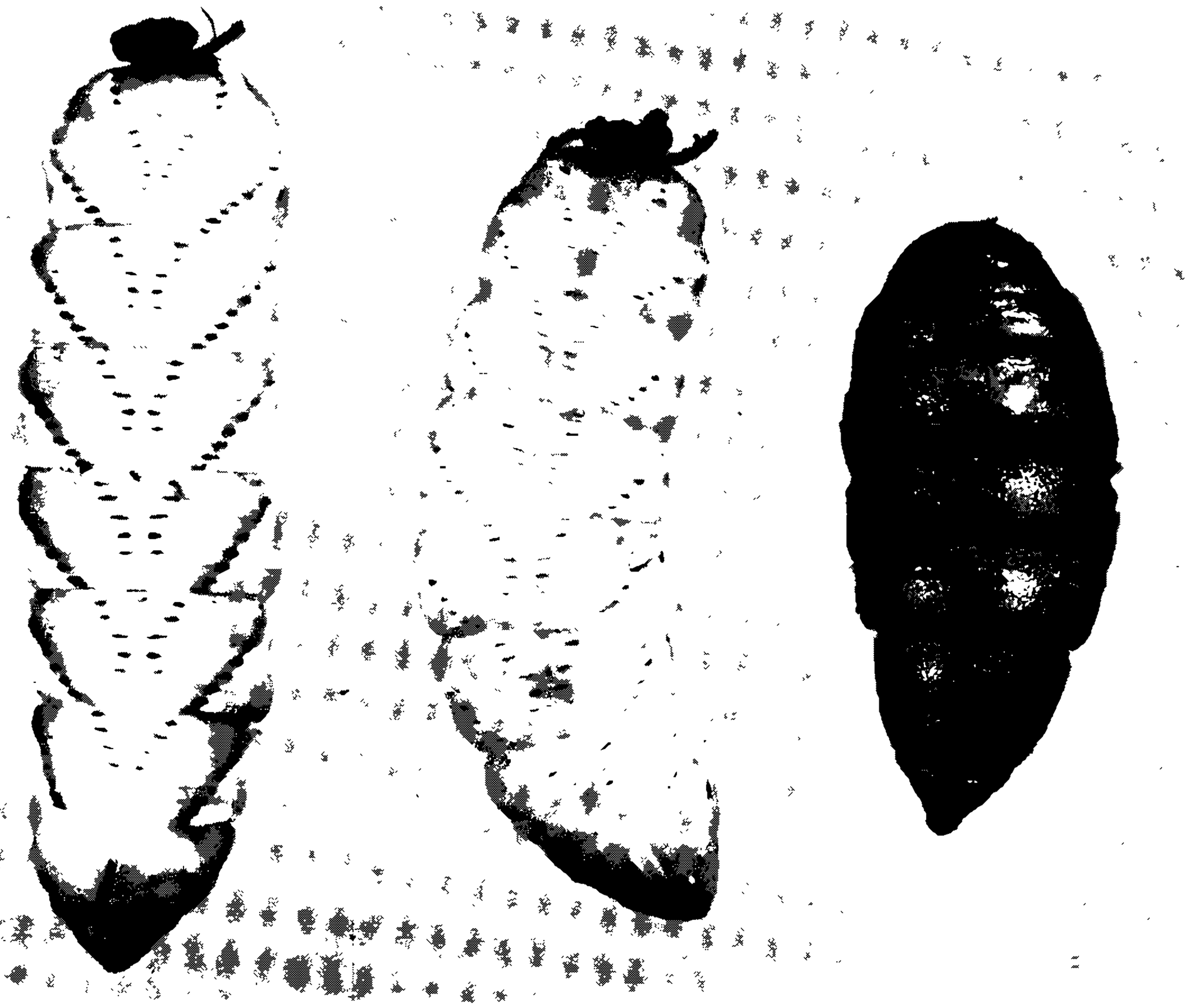
Extirpation of endocrine organs - a classic maneuver in hormonal research - has been difficult or impossible in the case of the prothoracic glands (PG) of insects. In larval and pupal Lepidoptera the glands are virtually inaccessible unless one sacrifices the insect. Even then, the PG are not easy to remove in their entirety. Consequently, in order to obtain viable preparations lacking PG, one customarily makes use of abdomens isolated by ligation or surgery.



These larval abdomens were isolated by ligation from feeding final (fifth) instar *Manduca* larvae. In the absence of PG the one on the left undergoes no further development. The one in the middle received implants of brain plus PG; it shows exposure of the dorsal vessel signaling the initiation of metamorphosis. Five days later the pupal abdomen of an implanted preparation is fully formed as in the abdomen on the right.

Experiments utilizing isolated abdomens are shown in Figure. Here three abdomens of final (fifth) instar larvae of the tobacco hornworm have been isolated by ligation. In the absence of PG the abdomen on the left undergoes no further development. The one in the middle received implants of brain plus PG; it shows the first signs of renewed development in terms

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of the blanching and clearing of the larval integument thereby exposing the pulsatile dorsal vessel. This is a tell-tale sign of the beginning of the so called "wandering period" signaling the initiation of metamorphosis. Five days later the pupal abdomen is fully formed and in many cases it succeeds in escaping from the old larval exuviae as illustrated by the abdomen on the right. The point I want to make in this figure is that larval abdomens are able to pupate only after they have been provided with a source of ecdysteroids.

The PG are located on each side of the anterior end just internal to the first pair of spiracles. Seven years ago, Dr. Sho Sakurai, then a postdoctoral associate of my laboratory, found that it was possible to extirpate the PG in their entirety provided that one used diapausing *Manduca* about two weeks after pupation. He first removed a rectangle of integument from the thoracic tergum of an anesthetized pupa. The entire pupa except for the prothoracic spiracles was then submerged in Ringer's solution. The main body of each PG was identified on each side nesting in its characteristic position just medial to the large tracheal trunk adjoining the prothoracic spiracle. It was gently teased free of its fine connections to surrounding tissues and then withdrawn by the aid of two forceps used in a hand over hand fashion to draw the remainder of the gland slowly from more anterior regions. It was then transferred to a black, Ringer filled dish and inspected under a dissecting microscope to confirm that the entire gland had been removed. The rectangle of integument was returned and sealed in place with melted wax. Dr. Sakurai, already excellent at microsurgery, became exceedingly proficient in performing PG-ectomy.

A startling finding was in store for us when we observed the behavior of the first few dozen of these PG-less pupae. The absence of the PG seemed inconsequential! After prolonged storage the preparations terminated pupal diapause and underwent adult development in synchrony with unoperated controls. And, like the controls, they promptly responded to the implantation of a non-diapausing brain by terminating diapause and initiating adult development. Furthermore, when the ecdysteroid titer was measured at the outset of adult development, its level was at least 20 times that typical of diapausing pupae. The conclusion was inescapable: unlike larvae, *Manduca* pupae contain a brain-sensitive source of ecdysteroids outside the PG (Safranek et al., 1986).

This conclusion was further examined in experiments performed on pupal abdomens isolated by ligation just behind the thorax immediately after pupation. Here again, the prompt onset of adult development in pupal abdomens of both sexes could be provoked by the implantation of a non-diapausing brain.

Manifestly, the picture here presented is strikingly different than that encountered 40 years ago in experiments performed on Cecropia pupae. The Cecropia pupa required the implantation, not only of brain, but also of PG. This result had been crucial since it led directly to the discovery of the brain-PG axis and the paradigm: *brain drives PG and the PG secrete ecdysteroids to drive the tissues.*

These findings were first published in 1947 and enlarged upon in 1952 (Williams, 1947, 1952). Thus in retrospect, it appears that if the experiment had been carried out on *Manduca* rather than Cecropia, the brain-PG axis would have been camouflaged by the presence in *Manduca* pupae of a brain-driven source of ecdysteroid outside the PG. For this very reason I have until recently viewed *Manduca sexta* as something of a maverick among insects: findings based solely on this species were not to be trusted until they had been checked out on Cecropia.

The story took a swift turn when Frantisek Sehnal, the well-known Czech entomologist, visited my Harvard laboratory some three years ago. In the course of our discussions Sehnal produced a reprint of a paper that he and his coworkers had published in 1981 but which, strange to say, I had never seen (Sehnal et al., 1981). I was particularly gratified to observe that they had included in this study no less than ten diapausing Cecropia pupae. But I was shocked to learn the results obtained on abdomens isolated from those ten. Merely by implanting brains they had caused all ten to terminate pupal diapause and undergo adult development. Implantation of PG was unnecessary!

I was baffled by Sehnal's result which differed so dramatically from mine. Could there have been some difference between the Cecropia pupal abdomens used by Sehnal and me?

"What did you do with the pupal midgut?" I inquired, recalling that this voluminous organ had presented a major problem in my surgical isolation of Cecropia abodomens.

Sehnal explained that he had used a sterile razor-blade to slice the pupa transversely between thorax and the first abdominal segment before sealing the latter to a plastic slip. The midgut therefore remained undisturbed in the isolated abdomen. By contrast, in my experiments the abdomens were surgically isolated at the tips of the pupal wings and thereby including only the terminal five segments. The midgut was carefully removed for the simple reason that there was no room for it in the truncated abdomen. The 1952 paper (Williams, 1952) presented a detailed description of these maneuvers.

These converging thoughts transformed dissident findings into fresh insights. Sehnal's results argue that, in Cecropia as well as *Manduca*, the pupal midgut constitutes a brain-sensitive, abdominal source of ecdysone. What this implies is that by excising the midgut from the isolated abdomens of Cecropia I had serendipitously eliminated the abdominal source of ecdysone which otherwise would have hopelessly complicated my experiment and prevented the discovery of the brain-PG axis.

Subsequently, these leads have been followed up in studies performed in collaboration with Dr. Louis Safranek and Ms. Mei-Ann Liu. We find that homogenates prepared from the walls of the pupal midgut of *Manduca* contain materials strongly cross-reacting with ecdysteroid antibodies in our RIA. This is peculiar to the tissue comprising the walls of the pupal midgut: no ecdysteroids could be detected in the contents of the midgut or in homogenates of other pupal abdominal tissues or organs.

The absence in *Manduca* larvae but presence in *Manduca* pupae of a rich depot of ecdysteroids may not be as bizarre as it seems at first glance. Pupae cannot feed and, unlike larvae, have no further intake of phytosterols. Yet additional ecdysteroids will be necessary to propel the pupal-adult transformation. Since insects cannot synthesize the sterol ring system (see Svoboda & Thompson, 1985), the supply of ecdysteroids or imediate precursors thereof must be carefully conserved. That being so, the strategy of sequestering ecdysteroids in the pupal midgut makes sense especially since the hormone depot can be mobilized by the brain. The whole affair can be regarded as an ingenious fail-safe device rather than as just another trick of nature.

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