the initiation of puberty in the rhesus monkey and that a period of induced normal pituitary and ovarian function in sexually immature animals does not necessarily entrain continued ovarian cyclicity. Furthermore, our findings suggest that puberty is normally initiated by the activation of hypothalamic mechanisms that control the pulsatile release of GnRH into the pituitary portal circulation. What causes this activation after a long period of prepubertal quiescence, however, remains to be elucidated.

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References and Notes

1. D. J. Dierschke, G. Weiss, E. Knobil, *Endocrinology* **94**, 198 (1974).
2. D. J. Dierschke, F. J. Karsch, R. F. Weick, G. Weiss, J. Hotchkiss, E. Knobil, in *Control of the Onset of Puberty*, M. M. Grumbach, G. D. Grave, F. E. Mayer, Eds. (Wiley, New York, 1974), p. 104; M. M. Grumbach, J. C. Roth, S. L. Kaplan, R. P. Kelch, in *ibid.*, p. 115; E. O. Reiter, H. E. Kulin, S. M. Hamwood, *Pediatr. Res.* **8**, 740 (1974); J. S. D. Winter, C. Faiman, F. I. Reyes, W. C. Hobson, *Clin. Endocrinol. Metab.* **7** (No. 3), 513 (1978).
3. T. M. Plant, L. C. Krey, J. Moossy, J. T. McCormack, D. L. Hess, E. Knobil, *Endocrinology* **102**, 52 (1978); E. Knobil and T. M. Plant, *Front. Neuroendocrinol.* **5**, 249 (1978).
4. E. Knobil, T. M. Plant, L. Wildt, P. E. Belchetz, G. Marshall, *Science* **207**, 1371 (1980).
5. D. L. Foster, *Biol. Reprod.* **17**, 586 (1977).
6. Y. Nakai, T. M. Plant, D. L. Hess, E. J. Keogh, E. Knobil, *Endocrinology* **102**, 1008 (1978).
7. The GnRH solutions for infusion (lots 26306 AL and 34414 AL) were prepared as described in (*6*).
8. The radioimmunoassays were as follows: LH and FSH: P. E. Belchetz, T. M. Plant, Y. Nakai, E. J. Keogh, E. Knobil, *Science* **202**, 631 (1978); estradiol: J. Hotchkiss, L. E. Atkinson, E. Knobil, *Endocrinology* **89**, 177 (1971), modified by the use of an antiserum specific for estradiol (GDN-244), provided by G. D. Niswender [for the characteristics of this antiserum see S. G. Korenman, R. H. Stevens, L. A. Carpenter, M. Robb, G. D. Niswender, B. M. Sherman, *J. Clin. Endocrinol. Metab.* **38**, 718 (1974)]; progesterone: R. L. Goodman, *Endocrinology* **102**, 142 (1978). The sensitivity of the steroid radioimmunoassays was 25 to 30 pg/ml for estradiol and 0.2 ng/ml for progesterone.
9. F. J. Karsch, D. J. Dierschke, R. F. Weick, T. Yamaji, J. Hotchkiss, E. Knobil, *Endocrinology* **92**, 799 (1973).
10. J. R. Clark, D. J. Dierschke, P. A. Meller, R. C. Wolf, *Biol. Reprod.* **21**, 497 (1979).
11. The observation that the estradiol-induced gonadotropin surge is not dependent on the presence of GnRH in the pituitary portal circulation during the surge is in accord with similar findings in stalk-sectioned monkeys [M. Ferin, H. Rosenblatt, P. W. Carmel, J. L. Antunes, R. L. Vande Wiele, *Endocrinology* **104**, 50 (1979)].
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Histolysis Initiated by "Lymph Gland" Cells of *Drosophila*

Abstract. *In late third-instar larvae and young pupae of* Drosophila hydei, *the "lymph gland" releases cells that migrate through the hemocoel and selectively attach to the basal laminae of transient larval organs. The lymph gland cells contain many inclusions—in particular, dense bodies, which appear to be secreted at the attachment sites. Since this process occurs before histolytic changes become apparent, it is suggested that the lymph gland cells initiate histolysis.*

In holometabolous insects, two contrasting processes occur concomitantly during metamorphosis: histolysis of the transient larval organs and histogenesis of the prospective imaginal organs. Although much is known about the development and histogenesis of imaginal disks, little attention has been directed toward the process of histolysis. It is still poorly understood.

Two different views have been advanced to explain histolysis of larval organs during metamorphosis. First, Pérez (*1*) described the breakdown of larval tissues in *Calliphora* as the result of a direct attack by phagocytic hemocytes. Second, in *Lucilia* (*2*), *Drosophila* (*3*), *Sarcophaga* (*4*), and *Calliphora* (*5, 6*), initial autolytic changes of the larval organs, including the fragmentation of muscles ("sarcolyte" formation), were described as occurring independently of hemocytes and before the latter invade the tissue.

In a recent review, Rizki (*7*) emphasized that the larval "lymph gland" of *Drosophila* is an organ of uncertain function. Srdić and Gloor (*8*) studied this organ in *Drosophila hydei* and concluded that its role is at the beginning of metamorphosis. In this report we show that the lymph gland plays an essential role in pupal histolysis.

Morphology and behavior of larval lymph gland cells were studied in *D. hydei* by light and electron microscopy, in vivo and after removal and transplantation of lymph glands labeled in vitro with [^{3}H]uridine (1 hour; 10 μCi per microliter of Ringer solution), followed by autoradiography of 1-μm Epon sections of host flies at different times after transplantation. The tissue was fixed and prepared for electron microscopy by standard techniques. To identify lysosomes, the test for acid phosphatase was carried out (*9*).

In *Drosophila* embryos (*10*) and newly hatched larvae (*8*), the lymph gland consists of a few cells on either side of the aorta behind the brain. Less than 1 day after hatching, three pairs of cell groups form. These grow slowly during larval life in a manner comparable to the growth of imaginal disks (*8*). (In *D. hydei* larvae we observed a 100-fold linear increase in the size of the lymph gland between the first and the late third instars.) Twenty hours before pupariation, the lymph gland begins to release its cells, called oenocytoids (*11, 12*). At the end of the larval stage, 10 percent of all cells floating in the hemolymph are oenocytoids (*8*). The release process continues until 2 days after pupariation, when all cells are liberated and the lymph gland disappears.

Inside the lymph gland, the cells form a relatively compact mass surrounded by a distinct basal lamina (Fig. 1A). All cell nuclei are euchromatic and contain a large nucleolus. The cytoplasm is characterized by a well-developed, rough endoplasmic reticulum, abundant Golgi elements, and two types of vesicles. Besides many electron-transparent vesicles, a strikingly large number of dense bodies are present. These show a strongly positive reaction in the acid phosphatase test, which indicates that they are lysosomes (Fig. 1C). The dense bodies are often arranged in rows and form extensive cisternae, which branch at their periphery. These structures are similar to the GERL (Golgi endoplasmic reticulum lysosomes) in granule-forming vertebrate secretory cells (*13*). In lymph glands of middle third-instar larvae, the same cells contain few, if any, dense bodies. However, small vesicles with dense cores are often present at the forming face of the Golgi stacks (Fig. 1B). Such vesicles are also observed in lymph gland cells at more advanced stages and in oenocytoids in the hemocoel. They seem to represent an early stage of dense-body formation.

Lymph glands from late third-instar larvae were labeled as before and injected into the posterior parts of larvae of the same age. Autoradiography of 1-μm Epon sections from the hosts enabled us to identify, in the hemolymph, single cells from the donors. About 10 hours after transplantation, labeled cells were present throughout the body cavity of each host. The labeled cells selectively attached to the basal lamina of transient larval tissues; for example, the abdominal and thoracic muscles, tracheae, and fat body (Fig. 1, D and E). In the hosts, the basal lamina of the muscles often ap-

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peared less rigid at the sites where a lymph gland cell had attached (Fig. 1F). The attached cells contained many dense bodies, electron-transparent vesicles, and large, irregularly shaped vesicles with a floccular, electron-dense content. The latter vesicles bulged out at the periphery of each cell, where they probably discharge their contents (inset in Fig. 1F). This suggests that stored lytic enzymes (including acid phosphatase), destined to attack the lamina and plasma membrane of the target tissue, are released when a target organ is contacted. Macrophages in vertebrates also discharge lysosomal enzymes on contact with immunologically recognized target surfaces (*14*). In an early pupa (11 hours after pupariation), we observed that the basal lamina was absent at most places along the larval muscles and that marked lymph gland cells were now attached directly to the plasma membrane of the target tissue (Fig. 1G). As the pupae grew older, fewer intact labeled lymph gland cells were present in our autoradiographs. Instead, there were numerous small fragments of cytoplasm and some almost cytoplasm-free nuclei. The fragments still showed the typical cytoplasmic structures of lymph gland cells, as is shown in Fig. 1. Since fragmentation of formerly ellipsoidal or spindle-shaped cells would increase the surface area of the cytoplasm, resulting in an increased rate of secretion, we assume that this phenomenon is part of the normal behavior of the cells. Thus it seems that in fulfilling their task, the cells disintegrate. Indeed, 2 days after pupariation, very few cells and cell fragments were observed in the hemocoel and the lymph gland itself in both control and experimental flies.

During metamorphosis of *D. hydei*, hemocytes begin to enclose and phagocytize muscle and other larval tissue fragments within 10 hours after pupariation. Our autoradiographs did not show any labeled phagocytic hemocytes. The labeled lymph gland cells were devoid of even small phagocytic vacuoles, such as were present in most hemocytes. Moreover, when dead yeast or ink was injected into third-instar larvae, the lymph gland cells failed to phagocytize any of these particles, which most of the hemo-

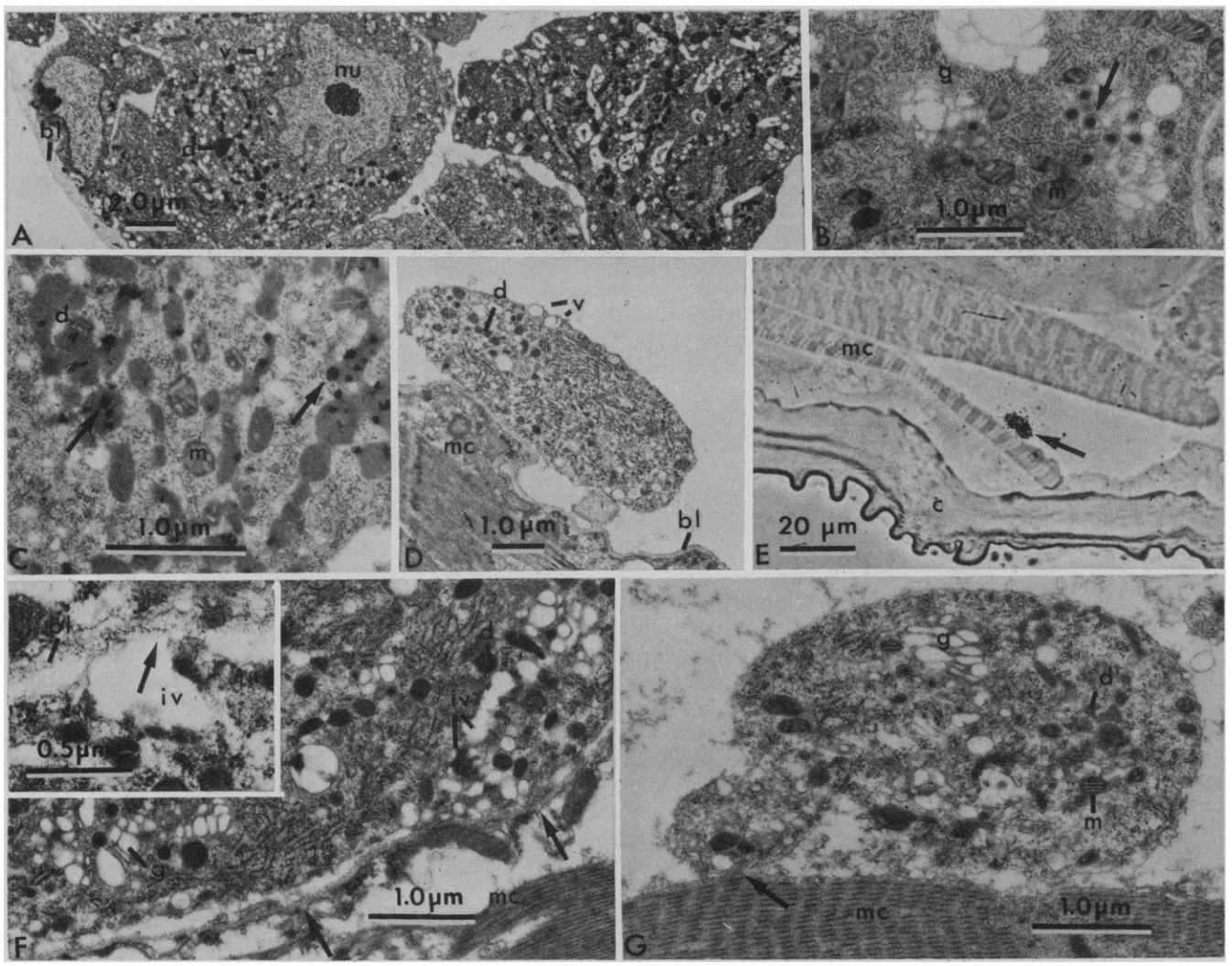

Fig. 1. (A) Cells at the periphery of a lymph gland in a late third-instar larva of *D. hydei*. Numerous dense bodies (*d*) and electron-transparent vesicles (*v*) are present in the cytoplasm (*bl*, basal lamina; *nu*, nucleus). (B) Vesicles with dense cores (arrow) at the forming face of the Golgi stacks (*g*) in the cytoplasm of a lymph gland cell in a middle third-instar larva (*m*, mitochondria). (C) Reaction to test for acid phosphatase activity in a lymph gland cell of a late third-instar larva. The reaction product (arrows) is specifically located over the dense bodies. (D) A labeled lymph gland cell (see E) attached to the basal lamina of a larval muscle (*mc*) at the time of pupariation (*v*, electron-transparent vesicles). (E) Light micrograph of a 1-μm section shows the identical labeled lymph gland cell (arrow) as in (D) (*mc*, larval muscle; *c*, cuticle). (F) Detail of a transplanted, labeled lymph gland cell that attached to a muscle cell of a late third-instar larva. The basal lamina of the muscle is discontinuous at some places (arrows). Inset: A large, irregularly shaped vesicle (*iv*) with a floccular, dense content ready to be discharged (arrow) toward the basal lamina of the target organ. (G) A labeled lymph gland cell attached to a larval muscle 11 hours after pupariation. The basal lamina along the muscle is absent and the lymph gland cell is attached directly to the plasma membrane (arrow).

cytes enclosed vigorously. Therefore, any participation of lymph gland cells in phagocytosis may reasonably be dismissed. Rather, we suggest that these cells are exclusively responsible for the initiation of histolysis. In this view, the organ would prepare larval tissues for the subsequent steps in histolysis, such as phagocytosis. The means by which the lymph gland cells recognize their target tissue and the nature of the signals triggering the release of lytic enzymes remain to be clarified.

Various roles have been attributed to the lymph gland (*7*). The present study would appear to rule out phagocytosis (*3, 15*). Nor did we find any support for melanin deposition in imaginal disk development of the eye, stimulation of imaginal primordia during metamorphosis, formation of the mesodermal parts of the imaginal appendages (*16*), and larval hematopoiesis (*17, 18*), thus confirming our previous conclusions (*8*). However, it is interesting to note that Gateff (*18, 19*) found, in a hereditary malignant blood neoplasm of *D. melanogaster*, *l(1)mbn*, that the lymph gland cells attack and histolyse the imaginal disk epithelia in situ. Moreover, when parts of such lymph glands were injected into normal adult hosts, various tissues were attacked, including flight muscles. These observations allow us to suggest that mutant lymph gland cells, in addition to having uncontrolled proliferation and possibly disturbed target specificity, may have increased histolytic potential.

In *Calliphora*, cells similar to the lymph gland cells of *Drosophila* were observed by Crossley (*20*) and by Zachary and Hoffmann (*21*). They also noted a strikingly large number of dense bodies with a strongly positive reaction in the acid phosphatase test. The "hematopoietic" organs in *Calliphora* (*20, 21*) or other Diptera [*Chironomus*, *Musca*, and *Phaonia* (*22*)] which release cells just before pupariation, could be organs analogous to the lymph gland of *Drosophila*.

Concerning the initiation of histolysis, our observations do not support the view of Pérez (*1*), who stated that hemocytes penetrate intact muscles. Nor do we agree with the concept of an autonomous autolysis of muscles during metamorphosis, as proposed for *Lucilia* (*2*), *Drosophila* (*3*), *Sarcophaga* (*4*), and *Calliphora* (*5, 6*). We propose that lymph gland cells are responsible for initiating histolysis of transient larval tissues before these are fragmented and phagocytized. Our observations point to a prehistolytic or clastic action of a specific type of cell as an indispensable first step in histolysis. Thus the term "lymph gland" should be changed according to the function of the organ upon confirmation of our prediction that similar histolytic organs will be identified in other holometabolous insects.

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References and Notes

1. C. Pérez, *Arch. Zool. Exp. Gen.* **4**, 1 (1910).
2. A. C. Evans, *Proc. R. Entomol. Soc. London Sect. A* **11**, 52 (1936).
3. C. W. Robertson, *J. Morphol.* **59**, 351 (1936).
4. J. M. Whitten, *J. Insect Physiol.* **10**, 447 (1964).
5. A. C. S. Crossley, *J. Embryol. Exp. Morphol.* **14**, 89 (1965); *J. Insect Physiol.* **14**, 1389 (1968); in (*6*), p. 499.
6. M. Ashburner and T. R. F. Wright, Eds., *The Genetics and Biology of* Drosophila (Academic Press, New York, 1978), vol. 2b.
7. M. T. M. Rizki, in (*6*), p. 397.
8. Ž. Srdić and H. Gloor, *Rev. Suisse Zool.* **85**, 11 (1978).
9. L. Ericsson and B. F. Trump, *Histochemistry* **4**, 470 (1964).
10. D. F. Poulson, in *Biology of* Drosophila, M. Demerec, Ed. (Wiley, New York, 1950), p. 168.
11. A. C. Hollande, *Arch. Zool. Exp. Gen.* **6**, 283 (1911).
12. C. J. Jones, *Am. Zool.* **2**, 209 (1962).
13. A. R. Hand and C. Oliver, *J. Cell Biol.* **74**, 399 (1977).
14. G. Weissmann, M. C. Finkelstein, J. Csernansky, J. P. Quigley, R. S. Quinn, L. Techner, W. Troll, P. B. Dunham, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1825 (1978).
15. A. Bairati, *Z. Zellforsch. Mikrosk. Anat.* **61**, 769 (1964).
16. H. H. El Shatoury, *Wilhelm Roux' Arch. Entwicklungsmech. Org.* **147**, 489 (1955); _____ and C. H. Waddington, *J. Embryol. Exp. Morphol.* **5**, 122 (1957).
17. M. B. Stark and A. K. Marshall, *J. Am. Inst. Homeopathy* **23**, 1204 (1930).
18. E. Gateff, in (*6*), p. 181.
19. _____, *Science* **200**, 1448 (1978); *Ann. Parasitol. Hum. Comp.* **52**, 81 (1977).
20. A. C. S. Crossley, *J. Exp. Zool.* **157**, 375 (1964).
21. D. Zachary and J. A. Hoffmann, *Z. Zellforsch Mikrosk. Anat.* **141**, 55 (1973).
22. L. Arvy, *Bull. Soc. Zool. Fr.* **78**, 158 (1953).
23. We thank B. Barandun for excellent technical assistance and H. Frei, H. Gloor, M. M. Green, and H. R. Kobel for critical comments on the manuscript. Supported by Swiss National Science Foundation grant 3.792-0.76.
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Brainstem Catecholamine Neurons Are Target Sites for Sex Steroid Hormones

Abstract. *Sex steroid hormones and catecholamines have physiological interactions in the brain. By the combined use of autoradiography and fluorescence histochemistry, steroid hormone target sites and catecholamine neurons were visualized simultaneously in the same tissue preparation. By this dual localization method, [^{3}H]estradiol and [^{3}H]dihydrotestosterone target sites were identified in nuclei of many catecholamine cell bodies in the brainstem, and catecholamine nerve terminals were observed near certain steroid hormone target neurons. These results suggest close anatomical interrelations between steroid hormone sites of action and catecholamine sites of production and action in the brain.*

Sex steroid hormones and catecholamines have physiological interactions in the central nervous system (CNS) (*1*) that appear to play a significant role in the regulation of a number of physiological processes, including ovulation (*2, 3*), copulatory behavior (*4*), and blood pressure (*5*). It is not known, however, where and how sex steroids and catecholamines interest in the CNS. In order to answer these questions, we developed a formaldehyde-induced fluorescence (FIF)-autoradiography technique that locates catecholamines and sex steroids simultaneously in the same tissue section (*6*).

Through autoradiography alone, estrogen and androgen target sites in rat brain have been located (*7*). Comparison of these steroid hormone target sites with catecholamine mapping data (*8*) suggests anatomical overlap between sex steroid target sites and catecholamine neuronal systems. In the present investigation, the FIF-autoradiography technique was used to locate concentrations of the female sex hormone 17β-estradiol and the male sex hormone 5α-dihydrotestosterone in relation to brain catecholamine systems in male and female rats. Estradiol was selected because its influence on CNS catecholamines is widely acknowledged; and dihydrotestosterone was chosen because it is an active testosterone metabolite that is not converted to estrogen and thus acts as a true androgen (*9*).

Two types of morphological relations between steroid hormone target sites and catecholamine neurons are described: (i) sex steroid hormones are concentrated in nuclei of catecholamine neurons and (ii) steroid hormone target neurons are surrounded by catecholamine terminals. This suggests that sex steroids directly influence catecholamine neurons by exerting genomic effects at nuclear sites (*7*) and that catecholamine neuronal systems influence steroid hormone target neurons by direct innervation of steroid hormone target neurons.

Six male and six female 60-day-old Holtzman rats were gonadectomized; the male rats were also adrenalectomized and

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