

endings are partially enclosed with the Schwann cell cytoplasm. The remaining axonal surfaces are separated from the surrounding connective tissue by only an external lamina, which passes from the Schwann cell cytoplasm on to the axon. In most cases, the endings lie more than 500 nm apart from the surface of the ganglion cells, but may approach more closely to the dendritic protrusions of the cells. This study, however, has failed to reveal the formation of typical synapses with the synaptic cleft approximately 200 Å in width and the density increases of synaptic membranes between the second type endings and the ganglion cells.

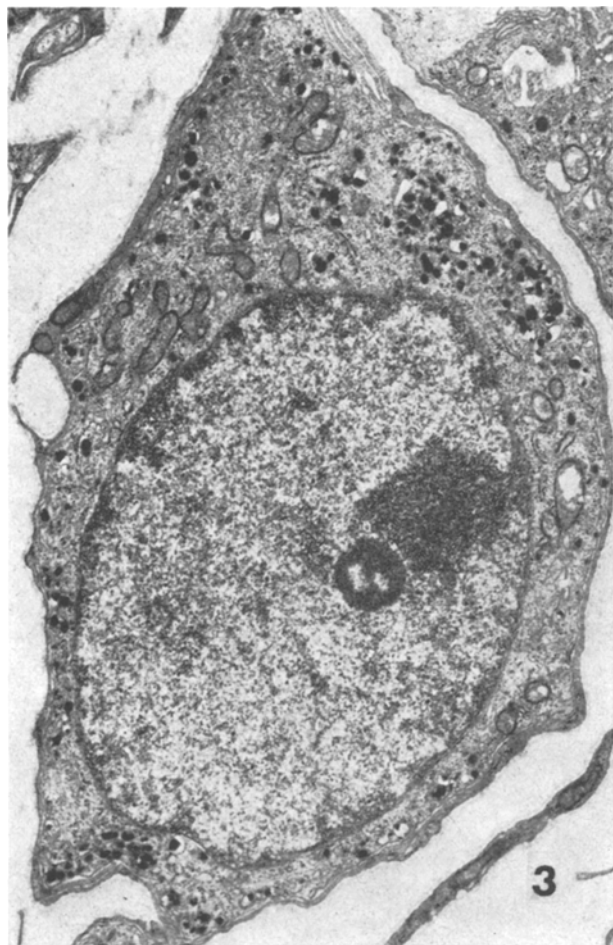


Fig. 3. Electron micrograph showing a cell containing large granular vesicles. The cell is surrounded by a sheath of satellite cells. $\times 6000$.

Small nerve cells which contain large granular vesicles measuring about 1250–2500 Å in diameter were found in the present study (Figure 3). The large granular vesicles show a similar appearance to the noradrenaline containing granules in the adrenal medulla. Recently, similar ganglion cells containing large granular vesicles were described in the sympathetic ganglion by some authors^{12,15}.

These small nerve cells may correspond to so-called chromaffin cells^{13,14} or small, intensely green-yellow fluorescent cells^{1,2} described by some authors. The somas are covered by the satellite cells. No axo-somatic synapses have been found around them. The nerve cells have a few processes which contain many large granular vesicles. Their distal ends denuded of the Schwann sheath often lie about 500 nm away from either the surface of ganglion cells or the capillary wall. These third type endings with the large granular vesicles are apparently different in structure and origin from the second type endings described before.

The observations reported above have furnished an adequate ultrastructural basis for the presence of adrenergic endings within the hypogastric ganglion. NORBERG et al.⁵ and KÄLMÄN et al.¹¹ suggested that the fluorescent endings are of an intraganglionic origin, and that they are formed by either interneurons or collaterals from the postganglionic adrenergic neurons. At least some of these fluorescent endings may be on the third type of this paper.

Zusammenfassung. Mittels elektronenmikroskopischer Untersuchungen wird die Anwesenheit adrenergischer Nervenendigungen im Ganglion hypogastricum des Meerschweinchens bewiesen.

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¹¹ B. CSILLIK, G. KÄLMÄN and E. KNYIHÁR, *Experientia* 23, 477 (1967).

¹² G. SIEGRIST, M. DOLIBO, Y. DUNANT, C. FOROGLU-KERAMEUS, FR. DE RIBAUPIERRE and CH. ROVILLER, *J. Ultrastruct. Res.* 25, 381 (1968).

¹³ CH. OWMAN and N. O. SJÖSTRAND, *Experientia* 22, 759 (1966).

¹⁴ CH. OWMAN and N. O. SJÖBERG, *Z. Zellforsch. mikrosk. Anat.* 74, 182 (1966).

¹⁵ L.-G. ELFVIN, *J. Ultrastruct. Res.* 22, 37 (1968).

The Larval Ring Gland of *Drosophila melanogaster*: A Comparison of Ebony and Oregon Strains by Use of a Qualitative Protein Stain

In *Drosophila melanogaster*, molting and metamorphosis are controlled by hormones from 3 endocrine structures: neurohormones produced by neurosecretory cells in the brain, ecdyson secreted by the prothoracic gland, and juvenile hormone from the corpus allatum¹. If juvenile hormone is present in abundance, the molt will be a larval molt; if it is present in small amounts or absent, the result will be a pupal or imaginal molt². Previous evidence³

suggests an increase in the activity of the prothoracic gland at metamorphosis, but it must be pointed out here that this apparent increase in activity may merely be compensating for the increased volume of the final instar larva⁴.

To study the protein content and, therefore, the activity of the ring gland we used the procedure of MAZIA, BREWER and ALFERT⁵. The larval molts of the Ebony

strain were compared to those of the Oregon strain. Serial sections of the ring gland were examined for histological and cytological information.

Materials and methods. 83 instar larvae were chosen and sexed on the basis of their morphological characteristics. The 2 strains were equally represented. A modification of the bromphenol blue technique according to MAZIA et al.⁵ was employed on paraffin embedded sections of the 2 strains of *D. melanogaster*.

Results. The cytoplasm, nucleus and nucleolus were observed for stain density. Respective concentrations of stain are not interpreted or discussed in this paper. Stain density for each area was estimated on the basis of 1–4 points; 1 point being least dense and 4 points being most dense. The points, representing degrees of stain intensity, were added up to a total arbitrary number of units which could be designated for the corpus allatum or the prothoracic gland of the ring gland. These data are summarized in Figure 1.

The prothoracic glands of both sexes of the 2 strains remained constant in apparent protein content from

second to third instar. The corpus allatum in both sexes of the Oregon strain decreased in activity and the corpus allatum of both sexes in the Ebony strain increased in activity from second to third instars.

It appears that the ring gland is zoned since there is greater activity in one central area. Zonation was found in Oregon female second instars and in both sexes of the 2 strains of the third instar. It was noted that median sagittal regions of these prothoracic glands or corpora allata showed greater protein content than the previous or subsequent 6 μ sections. It was this point of maximum protein content which was chosen to represent the prothoracic gland or corpus allatum of a particular age or sex (Figures 2 and 3).

Discussion. Since zonation was found in Oregon female second instars and in both sexes of both strains of the third instar stage, it can be seen that the zoned ring gland is characteristic of a more mature ring gland. Only the second instar Oregon female larvae have a zoned ring gland. Since previous investigators^{2,8–10} reported significant connections between the ring gland and sexual

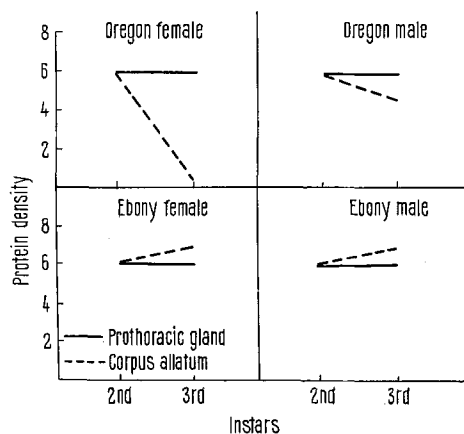


Fig. 1. Changes in protein density from second to third instar.

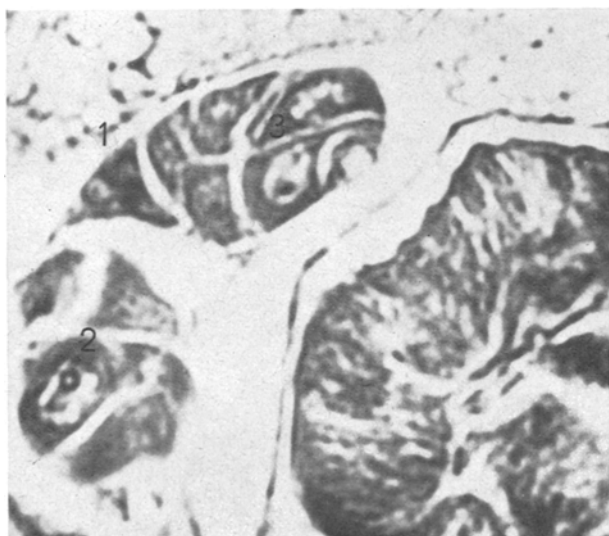


Fig. 2. Second instar Oregon female. Here the ring gland appears as a relatively homogeneous mass. (1) corpus allatum; (2) and (3) the 2 lobes of the prothoracic gland.

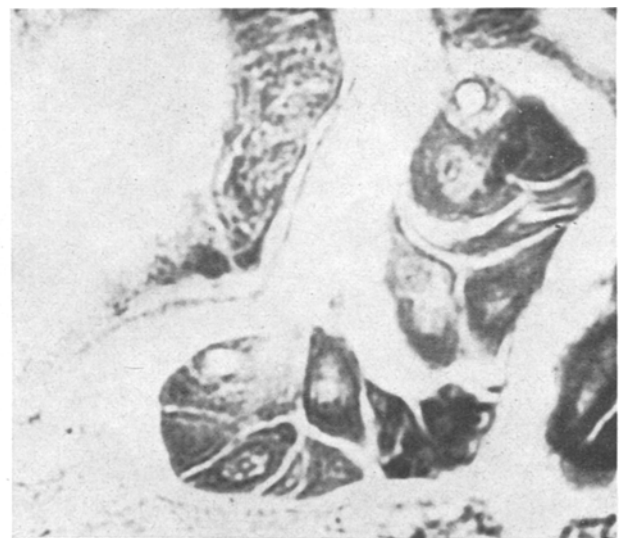


Fig. 3. Second instar Oregon female. This is one 6 μ section later. Note the more densely stained corpus allatum here as opposed to Figure 2. The next section found the ring gland once again homogeneous.

¹ L. I. GILBERT, in *The Physiology of Insecta* (Ed. M. ROCKSTEIN; Academic Press, New York 1964), vol. 1, p. 149.

² V. B. WIGGLESWORTH, *The Physiology of Insect Metamorphosis* (Cambridge University Press, Cambridge, England 1954).

³ V. B. WIGGLESWORTH, in *Advances in Insect Physiology* (Eds. J. W. L. BEAMENT, J. E. TREHERN and V. B. WIGGLESWORTH; Academic Press, New York 1964), vol. 2, p. 247.

⁴ H. A. SCHNEIDERMAN and L. I. GILBERT, *Science* 143, 325 (1964).

⁵ D. MAZIA, P. A. BREWER and M. ALFERT, *Biol. Bull.* 104, 57 (1959).

⁶ G. L. HUMASON, *Animal Tissue Techniques*, 2nd edn. (Freeman and Co., San Francisco 1967), p. 503.

⁷ M. J. NORRIS and M. P. PENER, *Nature* 208, 1122 (1965).

⁸ K. C. HIGHNAM, *Colln. Int. Centre Nat. Rech. Sci.*, Paris 114, 107 (1962).

⁹ K. C. HIGHNAM, *Insect Reproduction* (R. Entomology Soc., London 1964), p. 26.

¹⁰ K. C. HIGHNAM, L. HILL and W. MORDUE, *J. Insect Physiol.* 12, 977 (1966).

maturation, we can conclude that sexual maturation of the Oregon females begins earlier than in males and that Ebony females are late.

Results showed that the prothoracic glands of all stages, sexes, and strains remain unchanged. This conforms to the suggestion⁴ that the change in the activity of the prothoracic gland actually only accommodates growth of the larvae.

For the wild type, results conformed to previously reported data². We assume that the less active corpus allatum found consistently in our studies correlates with the decreased juvenile hormone titer. It would appear that with a less active corpus allatum, the ecdyson would be uninhibited and free to carry out the metamorphosis. This suggests that the juvenile hormone suppresses the activity of the prothoracic gland at second instar since the prothoracic gland activity was constant between second and third instars.

With respect to the Ebony strain, the corpus allatum increased in relative protein concentration from the second to the third instars. These results showed the prothoracic gland to be unchanged in protein activity from second to third instars. If the corpus allatum increased in activity and this is related to hormone activity,

there would be no molt. The hormone then is not being secreted to the circulatory system. Since there is still a larval molt, we know the corpus allatum is continuing to release juvenile hormone². As long as this juvenile hormone does not exceed the ecdyson level in the circulation, there will be a molt. Since the corpus allatum of the Ebony is so active, we can suggest that the corpus allatum is storing quantities of juvenile hormone or producing it at a greater rate¹¹.

Résumé. Le contenu protéique des cellules glandulaires circulaires des larves de 2 races de *Drosophila melanogaster* est examiné en détail. Les résultats de cette étude montrent que la production et l'activité hormonale de ces 2 races diffère.

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Influence of Hypophyseal Intermediate Lobe Tissue and Colloid on the Reticuloendothelial Cells of the Liver

Although a vast amount of literature has been devoted to the proliferation of reticuloendothelial cells of the liver under the influence of various agents¹⁻⁸, only a few doubtful mitotic figures, yet unpublished, have ever been observed in livers known to be synthesizing DNA⁹.

The dirth of even questionable mitotic figures in the face of experimental results indicating rapidly proliferating reticuloendothelial cells gives the impression that there may be either a recruitment of cells from other sources or an activation of cells potentially phagocytic.

An invasion of the liver with external macrophage precursors has been reported¹⁰, and furthermore, it is suggested that an increase in the number of actively phagocytic cells might be due to dormant cells rather than to mitosis of existing histiocytes¹¹.

In a continuing study of the effect of bovine hypophyseal substances on target organs, it was discovered that intermediate lobe tissue undergoing desquamation and autolysis as well as its resultant product, colloid, has a peculiar affinity for certain cells of mesodermal origin. Investigations showed that neither anterior nor posterior lobe tissue produced a similar effect.

In order to demonstrate the influence of intermediate lobe tissue and colloid on mesodermal tissue, reticuloendothelial cells of the liver proved to be a suitable model.

The liver of 8 adult dogs, both male and female were divided equally into 2 groups. 4 others were used as controls. The hypophysis from 2-year-old steers and heifers were collected within 15 min after slaughter and placed in Tyrode's solution for transport to the laboratory. After rinsing in several changes of Tyrode's solution, the glands were cut mid-sagittally into 2 equal halves, exposing the lobes of the gland and the residual lumen (intraglandular cleft). Only glands with a colloid filled lumen were utilized. The colloid was collected, and the avascular

intermediate lobe tissue, readily identifiable, was carefully scraped from the rostral surface of the posterior lobe. Neither intermediate lobe tissue nor colloid was pooled.

An inoculum was prepared by mincing separately intermediate lobe colloid and tissue into fine aggregates ($1/4$ mm or less) which were then divided into compartments 3 mm³. Each compartment contained approximately 29.6 mg/ml of protein. The inoculum from each compartment was fed into the pointed end of an 18-gauge hypodermic needle and the opposing end of the needle was fitted with a metal wire plunger.

The experimental animals were lightly anesthetized with ether and the inoculum was injected directly into the liver of each animal, transabdominally, below the twelfth rib in the right mid-axillary line. 2 male and 2 female animals received colloid, the others received intermediate lobe tissues. One animal from each group was sacrificed on day 3, 5, 7 and 9. By following the needle tract into the

¹ J. H. HELLER, R. M. MEIER, R. ZUCKER and G. W. MAST, *Endocrinology* 61, 235 (1957).

² D. L. J. BILBEY and T. NICOL, *Nature* 182, 674 (1958).

³ B. BENACERAF, B. N. HALPERN, G. BIOZZI and S. A. BENOS, *Br. J. exp. Path.* 35, 97 (1954).

⁴ E. R. GABRIELI and H. HOLMGRAN, *Acta path. microbiol. scand.* 31, 205 (1952).

⁵ J. G. HOWARD and A. C. WARDLAW, *Immunology* 7, 338 (1958).

⁶ N. R. DI LUZIO, *Am. J. Physiol.* 187, 595 (1955).

⁷ S. J. RIGGO and N. R. DI LUZIO, *Am. J. Physiol.* 200, 297 (1961).

⁸ T. NICOL and D. L. J. BILBEY, *Nature* 182, 192 (1958).

⁹ L. S. KELLY, E. L. DOBSON, C. R. FINNEY and J. D. HIRSCH, *Am. J. Physiol.* 198, 1134 (1960).

¹⁰ J. L. BOAK, G. H. CHRISTIE, W. L. FORD and J. G. HOWARD, *Proc. R. Soc. B.* 169, 307 (1968).

¹¹ J. G. HOWARD, *J. Path. Bact.* 78, 465 (1959).