THE NEUROSECRETORY SYSTEM OF THE FRUIT FLY *CHÆTODACUS CUCURBITÆ* Coq.

I. Distribution and Description of the Neurosecretory Cells in the Adult Fly

BY DR. K. K. NAYAR

(*Department of Zoology, University College, Trivandrum*)

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INTRODUCTION

NEUROSECRETORY cells, *i.e.*, nerve cells showing well-developed glandular activity, have been discovered in many groups of insects (*vide* Scharrer, 1954). These cells are situated in different parts of the central nervous system. Generally the insectan neurosecretory system has two parts: (i) Special cells of the protocerebrum and (ii) the axonic system forming the nervi corporis cardiaci and allati and the corpora cardiaca and allata. The corpora cardiaca and the corpora allata, originally described as the ganglia of the insectan sympathetic system, have recently been shown to be endocrine glands by various workers (review, Scharrer, 1948, 1953). In addition to the parts mentioned above, neurosecretory cells have been discovered in the frontal ganglion, sub-œsophageal ganglion, metathoracic and abdominal ganglia (Day, 1940; Scharrer, 1941; Nayar, 1953). The functional significance of the cerebral neurosecretory cells have been discussed by Scharrer (1952) in *Leucophæa maderæ*; by Thomsen (1952) in *Calliphora erythrocephala* and by Williams (1952) in *Platysamia cecropia.*

In this paper, an account is given of the neurosecretory cells seen in the brain, thoracic and abdominal ganglia of the fruit fly *Chætodacus cucurbitæ* Coq. (Trypetidæ: Diptera). A general description of the neurosecretory cells appeared to be desirable because of their specially characteristic distribution in the thoracic ganglion, forming a more conspicuous cluster than in the brain.

MATERIAL AND METHODS

Late pupæ (pupæ from which adults were about to emerge) and adult flies aged 1 to 4 days after emergence from puparia were used for study. The larvæ were reared on fruits of *Trichosanthus anguina* (snake gourd).

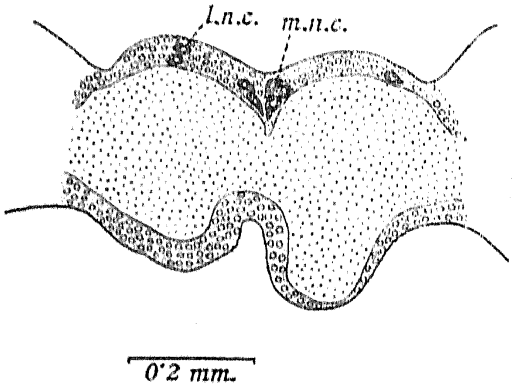
For examination of live cells, ganglia were dissected out in insect Ringer and were mounted on slides in the same solution. The cells continued

to live for more than an hour and were examined under the phase contrast and dark ground illumination.

The adults and pupæ (after removal from puparia) were fixed in Carl's, Carnoy's, Bouin's and Helly's fixatives. Material was double embedded by Peterfi's method using 1½ to 2% celloidin in methyl benzoate and sectioned at 5 and 8 to 10 μ. Heidenhain's iron hæmatoxylin, Groat's hæmatoxylin, Gomori's chrome-hæmatoxylin-phloxin and Heidenhain's Azan were the stains used. Gomori's method and Azan after Bouin fixation gave the best results.

Observations

Situated in the pars intercerebralis (Text-Fig. 1) is a cluster of about a dozen cells which show well-defined secretory activity. These form the



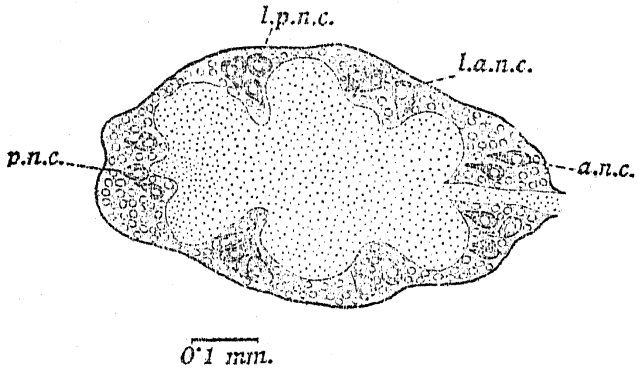
Text-Fig. 1. Camera lucida drawing (composite from a few adjacent serial sections) of a transverse section of the brain of adult *Chætodacus cucurbitæ* showing the median and lateral neurosecretory cells. *mnc.*, median neurosecretory cells; *lnc.*, lateral neurosecretory cells.

median cerebral neurosecretory cells. These cells have conspicuous axons traceable for a fairly long distance (Plate III, Fig. 1) and filled with secretory products. A few scattered cells in the lateral region of the brain form the lateral cerebral neurosecretory cells. These are situated near the region where the cerebral ganglia laterally carry the optic lobes. Both the sets consist of conspicuously large cells having large, more or less rounded nuclei with loosely arranged chromonemata and composite nucleoli stainable red in both Azan and Gomori's stains. The cytoplasm is coloured blue in chrome-hæmatoxylin and shows a reddish tinge in Azan due to the presence of numerous granules.

Large cells are distributed on the ventral side of the nerve ring also, but these do not show the blue granules in the cytoplasm. Here the granules are seen to be phloxinophil.

The nerve springing from the brain forming the nerves corporis cardiaci also is coloured blue due to the blue granules which could be traced into the corpus cardiacum below.

The neurosecretory cells of the thoracic ganglion are the most conspicuous ones (Plate III, Fig. 2). They show a definite arrangement in this composite ganglion. The single ganglionic mass is really composed of three ganglia fused together as is evidenced by the trilobed appearance of the neuropile in the centre. The neurosecretory cells are distributed along the margin of the ganglionic mass. There are four clusters (Text-Fig. 2):



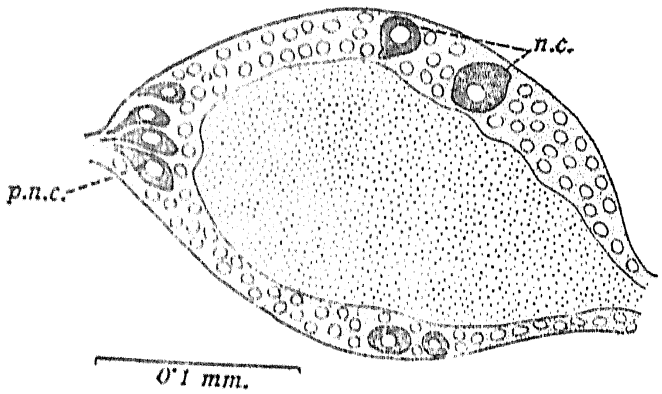
TEXT-FIG. 2. Camera lucida drawing (composite from a number of sections) of the horizontol section of the thoracic ganglion of the adult *Chætodacus cucurbitæ*, to show the distribution of the neurosecretory cells along the margin. *anc.*, anterior neurosecretory cells; *lanc.*, lateral anterior neurosecretory cells; *lpnc.*, lateral posterior neurosecretory cells; *pnc.*, posterior neurosecretory cells.

(1) an *anterior pair* of comparatively narrow, elongated cells arranged in two groups on the two sides of the connective from the sub-œsophageal mass; (2) a *posterior cluster* of cells forming a more or less continuous mass not quite separated into pairs, situated near the posterior end of the ganglion; (3) and (4) paired groups of comparatively larger cells placed laterally and in between the fibrous neuropiles (forming *lateral anterior* and *lateral posterior pairs*).

The nuclei of the thoracic neurosecretory cells are large, with characteristic chromonemata, chromocenters and nucleoli, which latter stain brilliant red in Azan. The cytoplasm is filled with granules which colour dark bluish-grey in chrome hæmatoxylin; in Azan they are reddish-dark grey. The granules which are blue or light blue extend along the axons also.

The neurosecretory cells of the single, small, abdominal ganglion are of special interest, because the few cells that are seen here show dark blue cytoplasmic products which have the appearance of colloids and fine

droplets and not quite that of fine granules (Text-Fig. 3). These cytoplasmic colloids show deeper blue colouration in chrome-hæmatoxylin-phloxin. A few are laterally placed and the other cells are lodged in the



TEXT-FIG. 3. Camera lucida drawing of a horizontal section (composite from a few adjacent sections) of the abdominal ganglion of adult *Chætodacus cucurbitæ*. *nc.*, neurosecretory cells; *pnc.*, postesior neurosecretory cells.

posterior part of the ganglion. The latter have the secretory products running into the nerve going posteriorly and this nerve in the sections show the blue granules among the fibres. These regions are probably the cut portions of the axons emerging from the neurosecretory cells. A main nerve supplies the mid and hind gut of the fly.

EXAMINATION OF LIVE CELLS

The thoracic ganglion when removed into Ringer's solution, is a composite single structure, and when gently pressed it becomes somewhat trilobed showing its three-segmented nature. It is not elongated, but more or less rounded and somewhat flattened. Associated with the ganglion are two conspicuous sac-like tracheæ which supply its lateral edges with numerous fine tracheoles. This tracheal supply indicates that the ganglion is particularly active in these regions. Such tracheal supply is usually seen in association with neurosecretory tracts.

The neurosecretory cells are large, more or less laterally distributed forming four clusters. They measure about 36μ in length and about 18μ in diameter in the swollen basal region. The larger cells may measure up to 60μ in length. The comparatively smaller cells anteriorly and posteriorly measure on an average 28μ in length. In course of time (after about 30 minutes) when cells are still alive, due to pressure and flowing movements of the cytoplasm, the cells become flattened or enlarged. Then the large cells may sometimes reach up to a length of about 90μ.

Under phase contrast, the cell shows densely granular cytoplasm, large rounded nucleus with a lower refractive index lying towards the large swollen (abaxonal) part, with dark, usually eccentric nucleolus. The cytoplasm shows dark granules and small vacuoles of varying sizes. These small vacuoles are sometimes clustered together. In the large cells, the vacuolar system of the secretory products are seen in the abaxonal part of the cell. Here they sometimes become contiguous and finally fuse and break off as a globule of secretory material. These vacuoles and droplets are more or less transparent and dark-rimmed. Smaller spheroids of a distinctly different type are scattered in the cytoplasm. The dark granules which probably represent the mitochondria are present in the droplets and vacuoles and in the cytoplasm proper of the cell.

In phase preparations, cells in connection with the nerve could be sometimes obtained. In preparations of the abdominal ganglion showing this connection, the cytoplasmic poducts from the neurosecretory cells are seen to flow into the nerve. The same picture could be obtained by examining the preparation under dark-ground illumination; here the cytoplasmic products which appear bluish-white and translucent are seen to move into the axons which run into the nerve.

DISCUSSION

The cerebral neurosecretory cells composed of the median and lateral clusters and the close association of the median ones with the corpus cardiacum in *Chætodacus cucurbitæ* resemble the condition observed in other dipterous insects (*vide* M. Thomsen, 1951; E. Thomsen, 1952, 1954; Scharrer, 1952; Possompes, 1953, 1954). Some workers have discovered the presence of the neurosecretory cells in the other ganglionic centres of the central nervous system. Scharrer (1941) has described the neurosecretory centres of the sub-œsophageal mass of the cockroach, Day (1940) has described the neurosecretory cells of the abdominal ganglia of Lepidoptera, and Nayar (1953) has observed the presence of these in the metathoracic ganglion of the bug *Iphita limbata.* The presence of conspicuous neurosecretory cells in definite tracts in the thoracic ganglion of *Chætodacus cucurbitæ* is very interesting. The distribution of these cells recall a resemblance to the large cells described by Matsumoto (1954) in the crab *Eriocheir japonicus.* In *Chætodacus* the axons from the thoracic cells could not directly be traced to nerves, but in certain cases they traverse the nueropile and end somewhat blindly. Whether these form neurohæmal clusters, as suggested by Carlisle and Knowles (1953) in the case of Crustacea, could be stated only after an intensive study of the nerve endings by special methods.

The neurosecretory cells of the abdominal ganglion in *Chætodacus* in the posterior region supply secretory products through the nerves, probably activating the mid and hindgut.

Summary

1. The neurosecretory system of the adult *Chætodacus cucurbitæ* (Trypetidæ: Diptera) consists of the cerebral, thoracic and abdominal centres of secretory neurons.

2. The cerebral neurosecretory centres consist of the median and lateral neurosecretory cells of the pars intercerebralis. The cells show characteristic tintorial properties when stained in chrome-hæmatoxylin-phloxin and in Azan, recalling the nature of similar cells in other insects.

3. The axons of the median cerebral neurosecretory cells are fairly long, and the nervi corporis cardiaci and corpus cardiacum show granules similar to those observed in the cytoplasm and axons of the cells.

4. The thoracic ganglion shows a more conspicuous set of neuro-secretory cells. They consist of an anterior, lateral anterior, lateral posterior and posterior cell clusters, which in the first three are paired.

5. The single abdominal ganglion contains a few neurosecretory cells also showing characteristic tintorial properties.

6. The nerve emerging from the abdominal ganglion and going to the gut shows blue granules traceable from the neurosecretory cells at the posterior end of the ganglion.

7. Phase contrast observations of the cells show that the neuro-secretory cells of the fly have a general structure with prominent cyto-plasmic products in the form of dark granules and large and small spheroids and globules.

8. Measurements of the thoracic neurosecretory cells, which forms the larger cells of the system, have been given.

Acknowledgments

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EXPLANATION OF PLATE

FIG. 1. Photograph of a longitudinal section of late pupa showing two median neurosecretory cells of the median tract in the brain. The axons are very well seen. Fixation Carnoy. Heidenhain's iron hematoxylin. Approx. × 1,000.

FIG. 2. Photograph of the longitudinal section along the margin of the thoracic ganglion of the adult fly showing the anterior and lateral anterior neurosecretory cells. Bouin fixed and stained in Gomori's chrome-hæmatoxylin-phloxin, × 180. Anterior cells to the left.

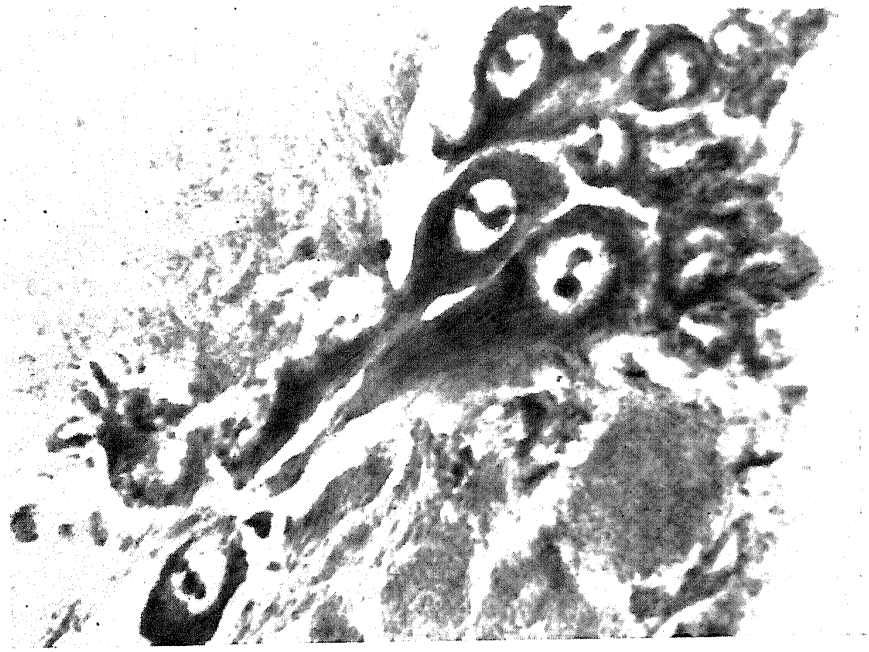

FIG. 1

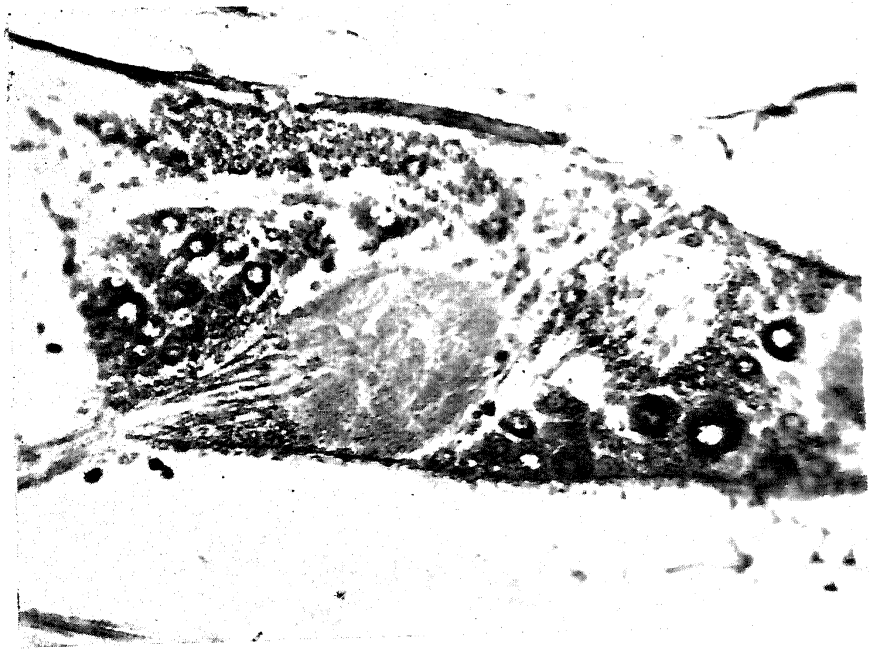

FIG. 2