Punjab Univ. J. Zool. Vol. 6, pp. 19-29, 1991.

HISTOLOGICAL CHANGES OCCURING IN SOME OF THE GANGLIA DURING METAMORPHOSIS OF THE VENTRAL NERVE CORD OF *PIERIS BRASSICAE* (LINN.) (PIERIDAE LEPIDOPTERA)

FIRDAUSIA AZAM ALI,

Department of Zoology University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan.

Abstract: Histrological changes occuring in the third abdominal ganglion during the metamorphosis of the ventral nerve cord of *Pieris brassicae* have been described in detail. Although these changes are extensive but third, fourth and fifth abdominal ganglion remain generally unchanged and resemble each other closely. These ganglia represent the typical structure as they retain their separate identity and are passed as such from the larva to the adult. The detailed structure of the third ganglion only has been described here while that of the fourth and fifth ganglia has been compared to it. The consistant features of this ganlion throughout the various postembryonic developmental changes include, two pairs of peripheral nerves to supply the segmental muscles of the body wall; a central "Structured Stratified type" of neuropile containing a number of glomerular regions and varying number of paired nerve tracts, and the cortex which consists of neurones and four types of glial cells.

Key words: Neuropile, axonal tract, postembryonis, development

INTRODUCTION

The investigation of the general histology of the insect nervous system has been largely directed towards the brain, with surprisingly little study of the ventral ganglia. Most of these accounts are morphological or microanatomical and are not greatly concerned with the detailed histology and metamorphosis of nervous system. An historical account of the central nervous system of insects is given by previous workers, *e.g.* Brandt (1879), Snodgrass (1935) and Wigglesworth (1965). No recent work has been done on histological aspects, although lot of work is present on physiology of the sensory organs (Lambin, 1984; Mitchel *et al.,* 1990, McVean, 1991., Vickers and Baker, 1991 and many others).

Among early workers Bauer (1904) and Beier (1927) dealt with the postembryonic development of the insect nervous system very briefly. Johansson (1957) also gave a brief account of the postembryonic development of *Oncopeltus*. Murray & Tiegs (1935), Cody and Gray (1948) and Sing & Srivastava (1973a,b) have described in more detail the postembryonic changes in *Sitophilus granaris*, *Philosoma ricini* and *Polistes hebraeus,* respectively. The work done on the postembryonic development of Lepidopteran ventral nerve cord is limited, but Pyle (1941), heywood (1965), Sing Srivastave (1973b) and Eaton (1974) have done some important work.

The objects and extent of the present study include a detailed histological analysis of the alterations which occur in the third, fourth and fifth ganglia of the ventral nerve cords and a description of the major axonal tracts and the neurone clusters which give rise to these tracts.

MATERIALS AND METHODS

First instar larvae of *Pieris brassicae* were kept constantly at 20 - 20 °C and fed on cabbage leaves. The various larval and pupal stages and the adults were reared from these. 5th instar larvae ready for pupation were also reared. The various larvae were killed for dissection and hitological treatment in the middle of the instar.

The vental nerve cord was removed by dissection in all cases except in the Ist and 2nd instar larvae whose size was too small to do this satisfactorily. Material was fixed in Bouin's solution and Zenker's or Gilson's fixatives for staining in Mallory's Triple Stain or Heidenhain's Iron Haematoxylin. The material was embedded in paraffin wax and serial sections were cut at 5 - 8 μm.

To study the nerve paths or tracts and to provide additional information on the cellular constituents, Wigglesworth's (1957, 1959) method of osmium tetroxide fixation followed by ethyl gallate treatment was used. Carlton and Drury's (1956) and Baker's (1966) account of osmium fixation was useful. material fixed in osmium tetroxide and stained by ethyl gallate was mounted in D.P.X.

RESULTS AND DISCUSSION

As the third, fourth and fifth abdominal ganglia remain unchanged in external appearance during the whole life of the insect, the histology of the third abdominal ganglion is first desribed in some detail so as to show its general constitution and the changes which occur postembryonically in its histological structure.

Peripheral Nerves

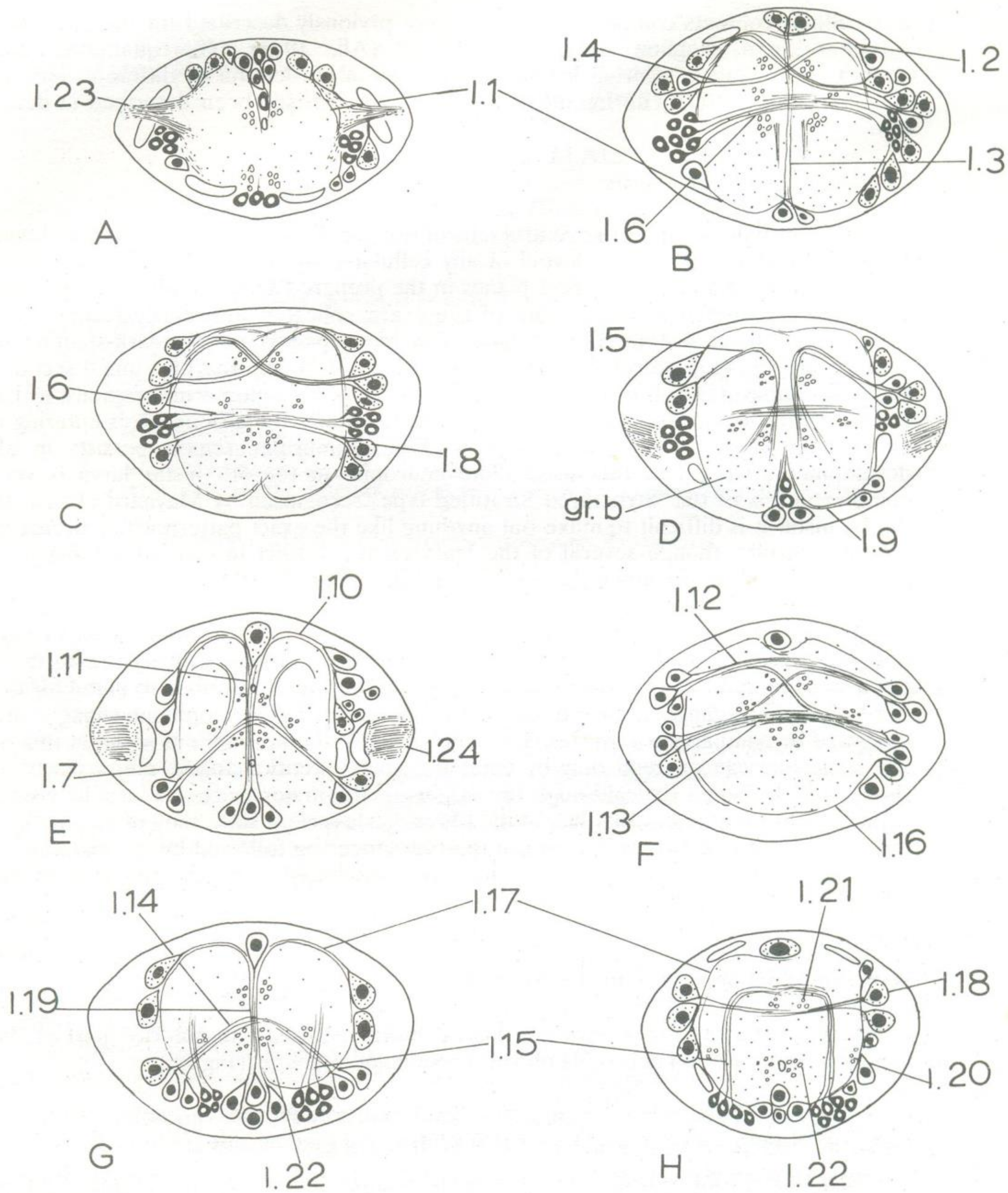
Two paris of peripheral nerves leave the third abdominal ganglion of every larval instar, prepupa and pupa to supply the muscles of the body wall. The anterior nerve runs transversely while the posterior one runs out lateroventrally. The axons which make up these nerves can clearly be seen leaving the neuropile in all stages except in the first instar larva where the nerves are small.

Cellular Contents

All the ganglion cells are peripheral, while the centre of the ganglion is occupied by neuropile which stains lightly with Heidenhain's Iron Haematoxylin and darkly by osmium-ethyl-gallate technique used originally by Wigglesworth (1959) and later on by Ali (1973). It is of the "Structured Stratified type" recognised by Maynard (1962).

Fig. 1. Serial diagramatic drawings of the transverse sections of the third abdominal ganglion of 5th inster larva showing neurones with their fibre tracts and other features. Cells with dark nuclei and dotted cytoplasm represent large motor neurones; cells with dark nuclei and white cytoplasm represent medium-sized neurones; cells with dark cytoplasm represent association neurones; small clusters of circles represent axons cut transversely and heavily shaded areas represent glomerular bodies.

Abbreviations used in figures: a.n., Association neurone; gl. I, Type I glial cell; gl.III, Type III glial cell; gl.IV, Type IV glial cell; gr.b, Glomerular body; m.n., Motor Neurone; nl., Neurilemma; np., Neuropile; pm., perineurium; tr., Trachea; tr. mt. c., Tracheal matrix cell; v. dm, Ventral diaphragm.


1.23
1.1
1.4
1.2
1.3
1.6
A
B
1.5
1.6
1.8
C
gr.b
D
1.9
1.10
1.11
1.12
1.24
1.7
E
1.13
F
1.16
1.14
1.17
1.21
1.18
1.19
1.15
1.20
G
1.22
H
1.22

The cellular contents comprise of 5 main types prviously described for the brain and suboesophageal ganglion of *Pieris brassicae* (Ali, 1973). The qualitative and quantitative changes occuring in them as well as in perilemma of different nervous ganglia of this butterfly during metamorphosis have already been described in detail (Ali, 1973, 1980).

Neuropile And Axonal Tracts

The neuropile occupies the central part of the ganglion. It includes occasional type IV glial cells but it is mostly devoid of any cellular component. It contains axons of different diameters cut in different planes in the prepared sections, but often running transversely or longitudinally. Some of these are collected into easily recognisable bundles and form distinct fibre treacts. The neuropile is mostly dark-staining in osmium-ethyl-gallate preparations and usually light in Haemotoxylin-stained sections (i.e. it is nonbasophil). It usually contains a number of pale glomerular regions, which are most conspicuous in the central part of the ganglion with various fibres entering it and breaking up into smaller branches. This glomerular region persists in all developmental stages of the insect. The neuropile in the 5th instar larva is very complex and is of the "Structured Stratified type" recognised by Maynard (1962). In the Ist instar it is difficult to make out anything like the exact pattern which occurs in the later instars, though several of the features are distinct in osmium-ethyl-gallate preparations. This difficulty is due to the samll size of the Ist instar.

In the 5th instar larva, where the ganglion is large and well differentiated, various fibre tracts can be made out clearly. In most cases the neurones giving rise to them can also be identified. The distribution of tracts is therefore described in detail for the third abdominal ganglion of the 5th instar larva and the development changes are discussed by comparison with this. The complexity of the neuropile is so great that at this stage one can proceed only by confining one's attention to the major tracts. It should also be noted that although the origins of the major tracts are usually visible without difficulty, the destinations of the fibres are less clear since they often pass into the undifferentiated neuropile and can than no longer be followed by the histological methods used here. The tracts described are numbered from the anterior to the posterior end of the ganglion.

The following are the paired tracts and other features of the neuropile of the third abdominal ganlion as seen in 5th instar larva:

Tract 1.1. From laterodorsal and lateral motor neurones in anterior part of the ganglion. Runs around neuropile on ventral side and medially (Fig.IA).

Tract 1.2. From laterodorsal and lateral motor neurones at anterior end of ganglion. Runs around neuropile towards ventral and median side (Fig.1A, B.

Tract 1.3. From ventral and dorsal association neurones at anterior end of ganglion, Runs in middle of ganglion dividing it into a right and left half (Fig.1A).

Tract 1.4. From laterodorasal motor neurones in anterior end of ganglion. Runs dorsomedially then curves downwards, going around periphery of neuropile, the two corresponding tracts from either side cross each other by a well defined chiasma in mid-dorsal line. Their fibres are lost along the margin of neuropile (Fig.1B, C).

Tract 1.5. From dorsolateral motor neurones towards anterior end of ganglion. Runs first towards the midline then curves down and runs medioventrally. Tracts of oposite sides run parallel ventrally towards ventral boundary of neuropile. Some of the fibres separate and curve outwards and are lost in a ventral glomerular body (Fig. 1 C,D).

Tract 1.6: From laterally placed clusters of association neurones. A prominent commissure which runs acros middline (Fig. 1 D).

Tract 1.7: From median sized ventrolateral motor neurones, Runs dorsally and medially very close to tract 1.6 and 1.8 (Fig. 1 D).

Tract 1.8: From laterally placed motor neurones. Runs across midline of ganglion in a more or less central position (Fig. 1 E).

Tract 1.9: From lateral and ventorlateral neurones in middle of ganglion. Runs across ventrally, encircling neuropile on the ventral side and forming its boundary in this region (Fig. 1 E).

Tract 1.10: From dorsolateral motor neurones. Runs dorsally encircling the neuropile in this region (Fig. 1 E).

Tract 1.11: From motor and association neurones lying on dorsal and ventral side in a median position in the middle of the ganglion. The ventral neurones send fibres upwards and dorsally placed neurones send fibres vertically downwards, while some of the fibres turn outwards and go around the neuropile. This tract forms a median partition dividing the neuropile into right and left halves (Fig.1 E).

Tract 1.12: From dorsolateral motor neurones in the middle of ganglion. Another commissure which runs across midline on dorsal side, dorsal to tract 1.13 (Fig. 1 E).

Tract 1.13: From a lateral group of motor neurones. Forms a commissure which runs across midline; some fibres run from it ventrally and are lost in a glomerular body, others are given off on its dorsal side and form a chiasma with the fibres from tract 1.12 (Fig. 1 E).

Tract 1.14: From ventrolateral motor neurones. Runs close to tract 1.13 forming a commissure (Fig. 1 G).

Tract 1.15: Form a well defined cluster of ventrolateral association neurones at posterior end of ganglion. Runs dorsalwards, passing over middline on dorsal side (Fig. 1 H).

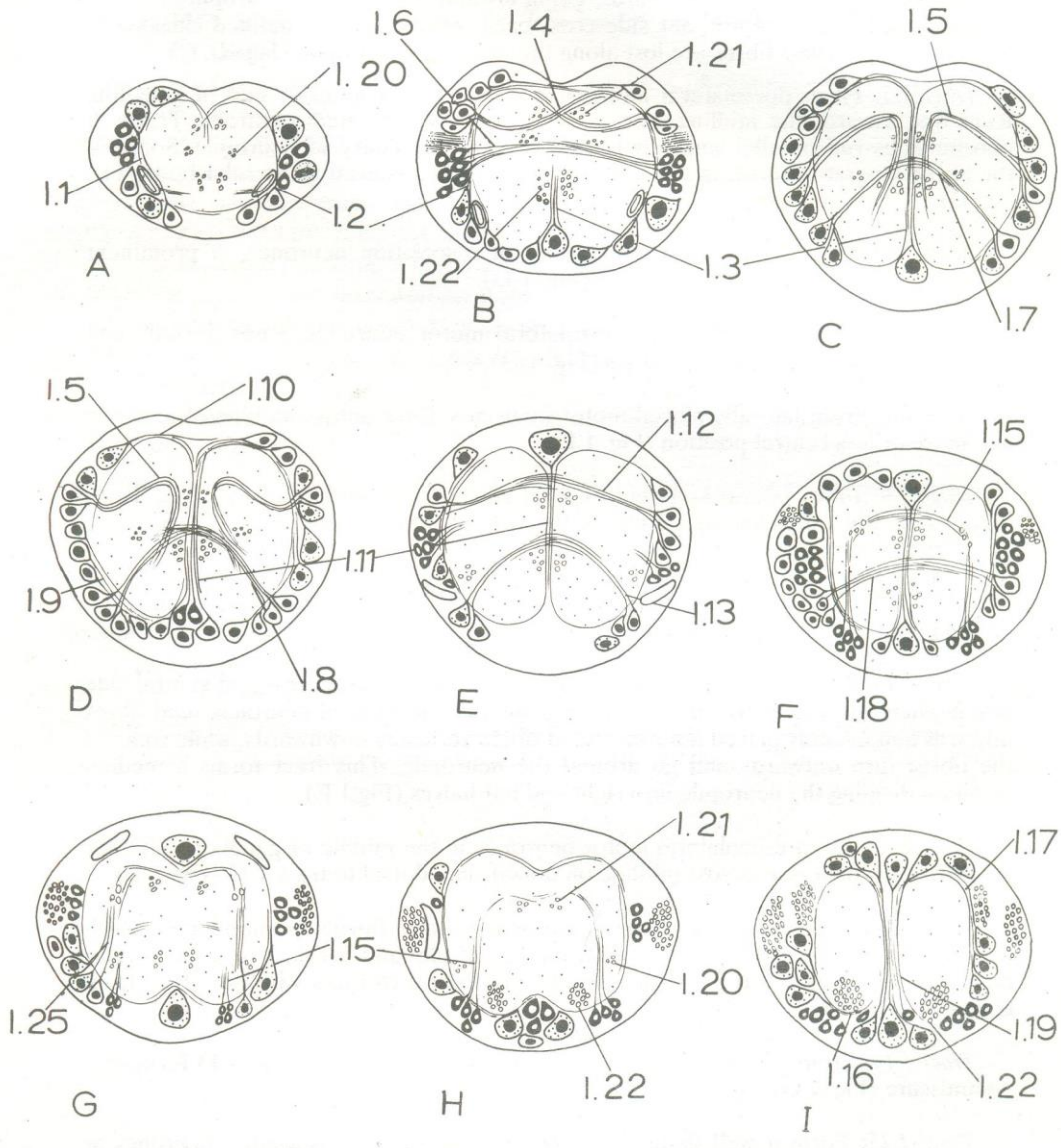


Fig. 2. Cross-section through posterior part of third abdominal ganglion of 250-hour puap showing motor neurones with their fibre tracts and glial cells.

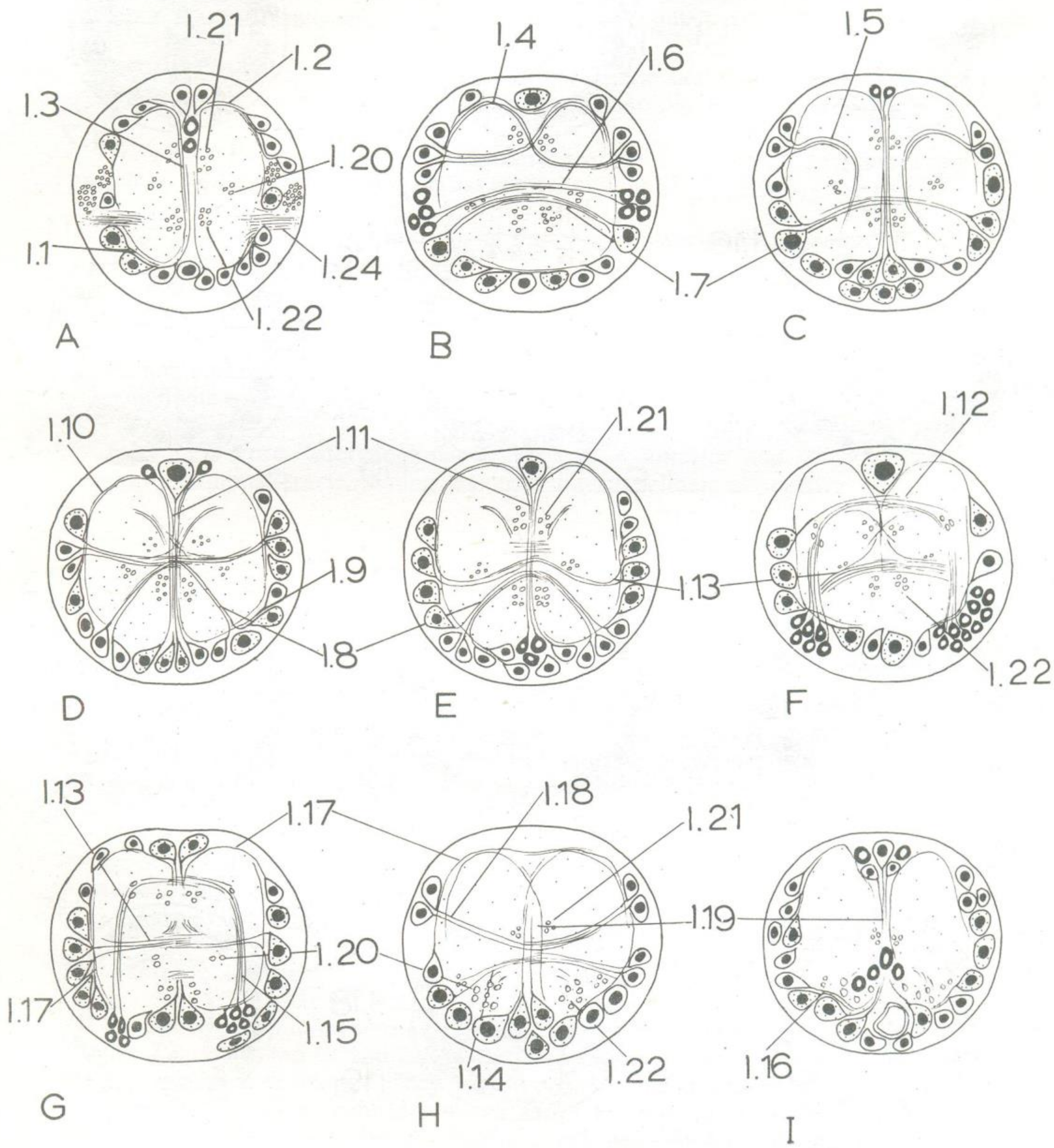


Fig. 3. Cross-section through posterior part of third abdominal ganglion of adult.

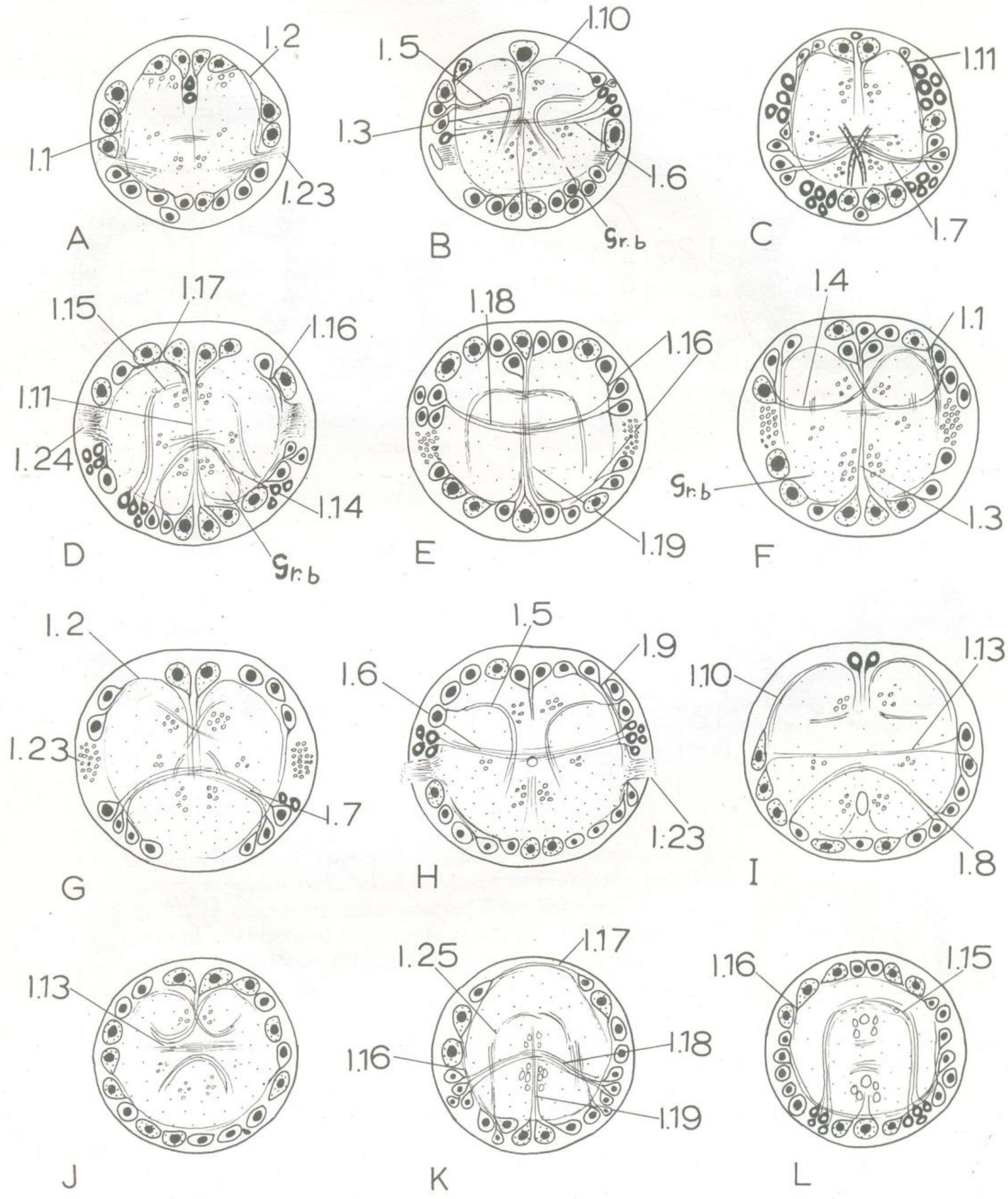


Fig. 4. Serial diagrammatic drawings of the transverse sections of the third abdominal ganglion of adult showing the same featues as Fig. 1 and with same explanations.

Tract 1.16: From lateroventral and laterodorsal motor neurones at posterior end of ganglion. Runs ventrally and medially encircling neuropile in this region (Fig. 1 G,H).

Tract 1.17: From lateral and dorsolateral motor neurones at posterior end of ganglion. Runs dorsally and follows boundary of neuropile in this region (Fig. 1 H).

Tract 1.18: From lateral motor neurones near posterior end of ganglion. Forms a prominent commissure running across ventral midline (Fig. 1 H).

Tract 1.19: From ventral and dorsal motor and association neurones. The fibres run vertically up and down in middle of neuropile and form a median vertical partition (Fig. 1 H).

Tract 1.20: Form interganglionic connective at anterior end of ganglion. Form paired longitudinal tracts running whole length of ganglion laterally, joining posterior integanglionic connective (Fig. 1 A, H).

Tract 1.21: Form interganglionic connective at anterior end of ganglion. Form paired longitudinal tracts, joining posterior interganglionic connective. (Fig. 1 A to H).

Tract 1.22: Form interganglionic connective at anterior and of ganglion. The two lonngitudinal tracts of opposite sides pass parallel to each other in midline. They traverse whole length of ganglion and join interganglionic connecitve at posterior end of ganglion (Fig.1 A, H).

Tract 1.23: From glomerular body on ventral side of ganglion; some fibres come from middle and dorsal side of neuropile and anterior interganglionic connective. A thick bundle of these fibres forms anterior peripheral nerve which leaves ganglion laterally. Some of these fibres run into anterior interganglionic connective (Fig. 1 A - D).

Tract 1.24: From dorsal and ventral part of neuropile and surrounding motor neurones. These fibres leave the ganglion ventrolaterally as second peripheral nerve (Fig. 1 F and G).

Postembryonic Changes

The above account of the tracts of the third abdominal ganglion refers only to the 5th instar larva. In the Ist and 2nd instar larvae the ganglion is vary small and not all the tracts are well developed. They are often feeble and the various cell groups, whch are so distinct in the later developoment stages, are not very well differentiated here, though they are much better distinguished in osmium-ethyl-gallate than in Heidenhain's Haematoxylin preparations. There is relatively little variation in size among the different motor neurones in these instars. In the 3rd and 4th instars, however, the neurones are clearly divisible into three size-classes and the various tracts are strong and distinct. The general appearance of the ganglion in these stages is the same in the 5th instar larva, except for the smaller size (Ali, 1980).

In the prepupa and pupa the conditions are also generally similar. The neurone-groups and the tracts arising from them are the same as in the 5th instar larva. But in the late pupa and adult, though the various tracts and the position of the various neurone-groups giving rise to them are similar (Figs. 2 and 3), the ganglion instead of being flattened is almost spheroidal. The association cell groups are not so prominent in the late pupa and adult through their tracts are very clear (Fig. 3). Fig. 1 and 4 show the comparable tracts in the 5th instar larva and the adult respecively very clearly. The size of the different cell nuclei is at a maximum during the prepupa and early pupa; later it decreases, parallel with the decline in the total ganglionic volume (Ali, 1980). The neuropile in the later pupal stages and in the adult is more compact and dense in appearance.

Fourth and fifth addominal ganglia

These two ganglia are of about the same dimentions as the third abdominal ganglion and all three resemble each other very closely, since they are the only ganglia which remain unchanged throughout postembryonic development, retaining their separate identity up to the adult stage. The various fibre tracts and neurone-groups giving rise to them are the same as in the third abdominal ganglion. Because of their close resemblance to the third abdominal ganglion they are not described here in detail.

REFERENCES

ALI, F.A., 1973. Postembrionic changes in the central nervous system and perilemma of *Pieris brassicae* (L.) (Lepidoptera: Pieridae). Trans. R. Ent. Soc. Lond. **124**: 463-498.

Ali, F.A., 1980. Some quantitative aspects of the metamorphosis of the central nervous system of *Pieris brassicae* (L.). *Pakistan J. Zool.*, **12**:99-114.

BAKER, J.R., 1966. *Cytological Technique*. London, 5th edn. 149 pp.

BAUER, V., 1904. Zur inneren Metamorphose des Centralnervensystems der Insection. *Zool. Ib. Anat.*, **20**:123-152

BEIER, M., 1927. Vergleichenide Untersuchungen uber den Centralnervensystems der Coleopteren larven. *Z. Wiss. Zool.* **130:** 174-250.

BRANDT, H., 1879. Vergleichend-anatomische Untersuchunngen uber das Nervensystems der Lepidoptera. *Soc. Ent. Res. St. Petersburgh*, **15:** 68-83.

CHARLTON, H.M. AND DRURY, R.A.B., 1956. Histological Techniques for Normal and Pathological Tissues and the Identification of Parasites. London. pp. 343.

CODY, F.P. AND GRAY, I. E., 1938. The changes in the central nervous system during the life history of the beetle, *Passalus cornutus Fabriciuus*. *J. Morph.*, **62:** 503 -522.

EATON, J.L., 1974 Nervous system of the head and thorax of the adult tobacco horn worm, **Manduca saxta** (Lepidoptera: Sphingidae). *Int. J. Insect Morphol. Embryol.*, **3**: 47-66.

HEYWOOD, R.B., 1965 Changes occurring in the central nervous system of *Pieris brassicae* (Lepidoptera) during metamorphosis. *Insect. Physiol.*, **2**: 413-430.

JOHANSSON, A.S., 1957. The nervous system of the milkweed bug, *Oncopeltus fasciatus* (Dallas): Heteroptera, Lygaeidae. *Trans. Am. ent. Soc.*, **83:** 119-183.

LAMBIN, M., 1984. Retinal scanning and visual inputs in freely walking crickets *Physioal. Entomol.*, **9**: 175-179.

MAYNARD, D.M., 1962. Organisation of neuropile. *Am. Zoologist.*, **2:** 79-96.

McVEAN, A. 1991. Some mechanical properties of the flagellar-pedicellar suspension in *Aedes aegypti* (L.), Physiol Entomol., **16**: 435-438.

MITCHELL, B.K., ROLSETH, B. M. AND McCASHIN,B.G. 1990. Differential responses of galeal gustatory sensilla of the adult colorado potato beetle, *Leptinotasa decemlineata* (Say), to leaf saps from host and non-host plants. *Physiol. Entomol.*, **15:** 61-72.

MURRAY, F.V. AND TIEGS, O.W., 1935. The metamorphosis of **Calandra oryzae Q.J. microse, Sci., 77:** 405-495.

SING, Y.N. AND SRIVASTAVA, U.S. 1973a. Anatomical changes in the nervous system of the castor silk moth, *Philosoma ricini* Hutt (Lepiodoptera: Saturniidae) during metamorphosis. *Int. J. Insect. Morphol. Embryol,* **2:** 169-175.

SING, Y.N AND SRIVASTAVA, U.S.,1973b. Histomorphological changes in the brain and nerve cord of the Indian wasp, *Polistes hebraeus Febr.* (Hymenoptera: Vespidae) during metamorphosis. *Morphol. Tiere.*, **75:** 125-135.

SNODGRASS, R.E. 1935. *Principles of Insect Morphology.*, New York and Lond. 667.pp.

VICKERS, N.J. and BAKER, T.C. 1991. The effect of unilateral anteectomy on the flight behaviour of male *Heliothus virescens* in a pheromone plume. Physiol. Entomol. **16**:497-506.

WIGGLESWORTH, V.B. 1957. The use of Osmium in the fixation and staining of tissues. *Proc. R. Soc. Lond.*, **147:** 185-199.

WIGGLESWORTH, V.B. 1959. The histology of the nervous system of an insect, *Rhodnius prolixus* (Hemiptera). (i) The peripheral nervous system. (ii) The central ganglia. *Q. J. microsc. Sci.,* **100:** 285-313.

WIGGLESWORTH, V.B. 1965. *The Principles of Insect Physiology.*, London, 6th edn. 741.pp.