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# Studies on the Neurosecretory System in Apterygota
## I. Histological Observation on the Corpus Allatum and Neurosecretory Cells in *Ctenolepisma*

By

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A wealth of studies have been carried out concerning the corpus allatum of the pterygotous insects from the cytological, histological and also experimental points of view. But so long as the apterygotous insects are concerned, no literature is available, except for those of HANSTRÖM (1940, 1942) and CAZAL (1948). CAZAL described the occurrence of the neurosecretory cells and the corpus allatum in some apterygotous insects, especially in *Japyx*.

A few years ago I had a lucky chance to find the corpus allatum of a Japanese silverfish, *Ctenolepisma villosa*, when I was studying its neurosecretory system. The present paper deals with the cytological and histological observation of this organ and the neurosecretory system alone, the physiological function of the corpus allatum being described elsewhere.

I wish to express my hearty thanks to Prof. Mamori ICHIKAWA, the University of Kyoto, for suggesting this problem and for critical reading of the manuscript. Thanks are also due to Prof. Iwane SATO of the Osaka University for his helpful advice.

### Material and Methods

The adults of *Ctenolepisma villosa* measure 9 mm long on the average. They live mostly on the starched paper and are found in book-cases and store-rooms all the year round. The specimens were each cut into the anterior and posterior halves in order to facilitate rapid penetration of the fixative. As the fixative BOUIN's or CARNOY's fluid was mostly used. After fixation, the specimens were immersed in methylbenzoate-celloidin mixture[1] for about 48 hours (DEMEREC, 1950) in order to soften the chitinous hard parts of the body. Sections were cut at 5 micra, transversely and longitudinally, using the routine paraffin technique, and stained, in general, with DELAFIELD's haematoxylin and eosin.

1) Methylbenzoate plus celloidin (99 : 1).

But GOMORI's chrom-haematoxylin and phloxin stain (GOMORI, 1941) was also applied to the material fixed in BOUIN's fluid, after oxidation in permanganate solution. This stain was proved suitable to reveal the neurosecretory substances in the cells.

## Observation

*Neurosecretory cells.* There occur two kinds of neurosecretory cells in the brain, one is the medial neurosecretory cells and the other lateral neurosecretory cells.

The medial neurosecretory cells are divided into two groups, both of which are found on either side of the median plane of the protocerebrum. One group is situated on the medial, dorsal surface of the brain as a small protrusion that is composed of about fifty cells (MNCl in Figs. 1 and 2a). The protrusion is encapsuled with a connective tissue. The cells are spherical about ten micra in diameter, and some of them are found to contain, in cytoplasm, many granules which are stained deep blue with chrom-heamatoxylin (Fig. 2b). These neurosecretory cells send a nerve to the brain as is indicated with an abbreviation of N1b in Fig. 2a. The other group lies amidst the cells of brain near the entrance of this nerve into the brain, and it comprises only two acidophilic cells (MNC2 in Figs. 1 and 3).

A fine nerve coming from these cells joins together with the nerve just mentioned to make the medial nerve which crosses the same nerve of the opposite side on the way to the post-ventral part of brain, where it leaves the brain to innervate the corpus cardiacum and dorsal aorta (N1 in Fig. 1).

The lateral neurosecretory cells can be distinguished as a group of three cells which are situated some 50 micra apart from the median plane of the brain (LNC in Figs. 1 and 5). Each cell is large, measuring 25 micra in diameter. The cytoplasm of it is unstainable with both of the acidic and basic dyes, and devoid of any kinds of droplets and granules. The nerve fibers of these cells leave the brain from the post-ventral part of the same side for the corpus cardiacum. This corresponds to the lateral nerve of other insects (N2 in Fig. 1).

*Aortic nerve and corpus cardiacum.* The medial and the lateral nerves on each side gather together immediately after they leave the brain. This composite nerve may be referred to as nervus corporis cardiaci, but the main destination of it is not the corpus cardiacum but the aorta. Therefore, it will be better to call it the aortic nerve. The aortic nerve runs backwards, through the corpus cardiacum, along the lateral wall of the aorta up to the junction point of the aorta and the heart. The corpus cardiacum receives fine fibers ramified from this nerve within it. It must be stressed here that many conspicuously stained blue droplets and a few red ones are found in this aortic nerve. This fact suggests that the aortic nerve serves as a pathway of the neurosecretory substances produced in the neurosecretory cells in the brain.

The corpus cardiacum is situated immediately behind the brain. It is a spherical body about 30-35 micra in diameter and is composed of some twenty cells, the cytoplasm of which shows very poor affinity for both basic and acidic dyes. Stainable droplets can not be seen in and between the cells (Fig. 6). The situation seems to differ much from that of the winged insects in which many droplets can be demonstrable in various species.

*Corpus allatum.* The position of corpus allatum is rather different from that of Pterygota so far informed. This organ is located at the base of each

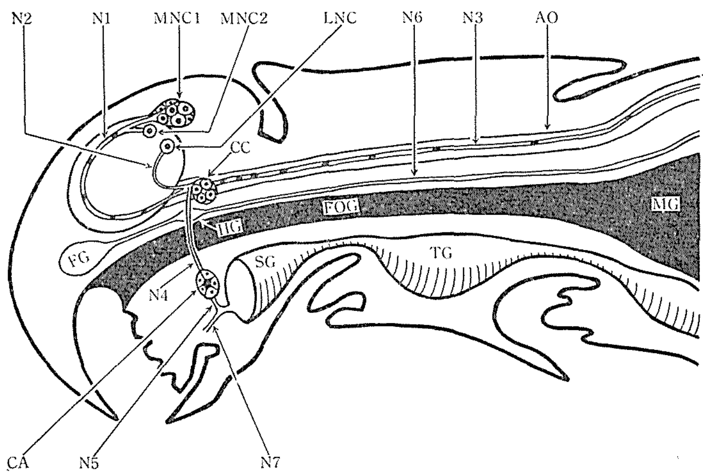


Fig. 1. Diagram of the neurosecretory system and corpus allatum in *Ctenolepisma villosa.*

MNC1, medial neurosecretory cells in a protrusion of the brain; MNC2, medial neurosecretory cells imbedded in the brain tissue; LNC, lateral neurosecretory cells; CC, corpus cardiacum; CA, corpus allatum; AO, aorta; N1, medial neurosecretory nerve; N2, lateral neurosecretory nerve; N3, aortic nerve; N4, nervus corporis allati; N5, an upper branch of the maxillary nerve; N6, gastric nerve; N7, maxillary nerve; FG, frontal ganglion; HG, hypocerebral ganglion; FOG, fore-gut; MG, mid-gut; SG, suboesophageal ganglion; TG, thoracic ganglion.

maxilla as a small body of about 35 micra in diameter (CA in Figs. 1, 7 and 8a). It consists of about fifty cells arranged into a vesicle. Sometimes the cavity of the vesicle is filled with a colloidal substance which takes blue dye when GOMORI's chrom-haematoxylin and phloxin stain is applied. The cytoplasm of the cells is stained red with phloxin, and contains neither vacuoles nor granules whithin it (Fig. 8b).

The corpus allatum is innervated by two nerves. One is, as alluded in the above comment, a small branch of nervus corporis cardiaci, separating itself near the point where the medial and lateral neurosecretory nerves join together. This nerve runs through muscles to reach the surface of the corpus allatum (N4 in Fig. 1), and, therefore, it may be equivalent to nervus corporis allati of the other insects. Indeed, in one case a small droplet stained blue is observed distinctly in this nerve (Fig. 9), although such droplets were not observed in other cases. The other nerve of the corpus allatum is an upper branch of the maxillary nerve which comes from the suboesophageal ganglion (N5 in Fig. 1). These two nerves distribute over the surface of the organ and do not enter within.

## Discussion

It has been revealed by the present observation that the medial neurosecretory cells of an apterygotous silverfish, *Ctenolepisma villosa*, are differentiated into two groups, one group makes a small spherical body, protruded from the brain surface, and the other group is imbedded in the brain tissue. This situation of the medial neurosecretory cells bears a close resemblance to the appearance of the same cells in the other apterygotous insects, *Petrobium maritima*, reported by CAZAL (1948), but differs much from that of most kinds of the pterygotous insects in which the cells are always found imbedded in the protocerebrum. According to my embryological investigation of the present insect that will be published in a separate paper, the protrusion of the neurosecretory cells can not be seen until a few days after hatching. It is, therefore, evidently a post-embryonic formation.

No knowledge of the functional role of the lateral neurosecretory cells of this wingless insect has yet been gained. As is already mentioned, there are no visible signs which demonstrate the secretory function of the cells because of the lack of any stainable materials within the cytoplasm and the lateral nerve as well. Since, however, the lateral nerve is a part of the aortic nerve and runs backwards together, it is likely, but not proved, that there is a functional relationship between this group of cells and the neurosecretory system. CAZAL (1948) has already reported a quite similar topography of the medial and lateral neurosecretory nerves in *Petrobius* and *Lepismina*. The fact that both the blue and red droplets in the aortic nerve diminish themselves gradually as they travel backwards seems to indicate these stainable substances are discharged through this nerve into the aorta on the way of their rearward migration. But it is not sure whether the neurosecretory material is stored in each nerve terminal ending and then released or it is liberated before it reaches the terminal ending. This may be a device to fit for the lack of the intimate connection between the corpus cardiacum and the neurosecretory nerve, or contrarily, it will be the original form to release the secretory material before the storage function of the corpus cardiacum does develop in future phylogeny.

At any rate, the fact that the stainable substance cannot be detected in the corpus cardiacum in *Ctenolepisma* will favour either view.

In most pterygotous insects, the corpus allatum is situated in the region dorsal or lateral to the oesophagus, whereas in *Ctenolepisma* the organ is located at the base of maxilla, as is already mentioned. It is due, in my opinion, to the fact that the ectodermal rudiment of the corpus allatum invaginated first from the maxillary base can not migrate inwards, but it stays close to its mother layer, in spite of the same origin as in the pterygotous insects. It seems quite probable that this exceptional location of the organ has concealed itself from discovery for a long time until now. The colloidal substance in the central cavity of the corpus allatum seems to be secreted from the surrounding cells. We lack the evidence enough to assume that the colloidal substance in the central cavity is an accumulation of the neurosecretory material transferred via axons from the neurosecretory cells in the brain, as it is the case in some Lepidopterous insects (Ichikawa *et al.*, 1959). I am rather inclined to surmise that it will be equivalent to the juvenile hormone secreted from the allatum cells themselves. However, there remains a possibility that there is some relationship between the neurosecretory cells and the corpus allatum, since the two are connected, as in pterygotous insects, by a small branch of the neurosecretory nerve.

**Summary**

1) The corpus allatum and the neurosecretory system of *Ctenolepisma* were studied from the histological view point.

2) The medial neurosecretory cells on each side are divided into two groups; one is a small protrusion of the surface of brain and the other is imbedded in the brain tissue. Lateral neurosecretory cells are distinguished as a group of three large cells unstainable with basic and acidic dyes.

3) The nerve fibers of the medial and lateral neurosecretory cells join together shortly after leaving the brain and run backwards along the lateral wall of the aorta.

4) The corpus cardiacum is a small spherical body situated behind the brain. It is innervated by some fine nerve fibers branching from the aortic nerve, when the latter passes through this organ. No droplets can be seen between the cells of this organ.

5) The corpus allatum is also a spherical body, situated at the base of maxilla. It is innervated by two nerves; one is a branch of the neurosecretory nerve and the other is a branch from the suboesophageal ganglion. It is difficult to trace the neurosecretory material to this organ.

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## Explanation of Plate I

Fig. 2a. Longitudinal section of the brain, cut somehow obliquely through the protrusions of both sides. MNC1, medial neurosecretory cells; N1b, nerve fibers of MNC1.

Fig. 2b. One enlarged cell of MNC1 showing many blue droplets in the cytoplasm.

Fig. 3. Longitudinal section of the brain showing the medial neurosecretory cells imbedded in the brain tissue and fusion of nerve-fibers coming from the respective group of the neurosecretory cells.
MNC2, medial neurosecretory cells; N1a, a fine nerve of MNC2; N1b, nerve of MNC1.

Fig. 4. Transverse section of the brain showing the lateral neurosecretory cells.
LNC, lateral neurosecretory cells.

Fig. 5. Transverse section of the head showing the cut ends of medial and lateral nerves ready to join together.
N1, medial nerve coming from MNC1 and MNC2; N2, lateral nerve arising from LNC; AO, aorta; B, brain; SA, dorsal salivary gland; FOG, fore-gut.

Fig. 6. Transverse section of the head showing both of corpora cardiaca.
N3, aortic nerve with many stained droplets; AO, aorta; CC, corpus cardiacum; B, brain; HG, hypocerebral ganglion; FOG, fore-gut.

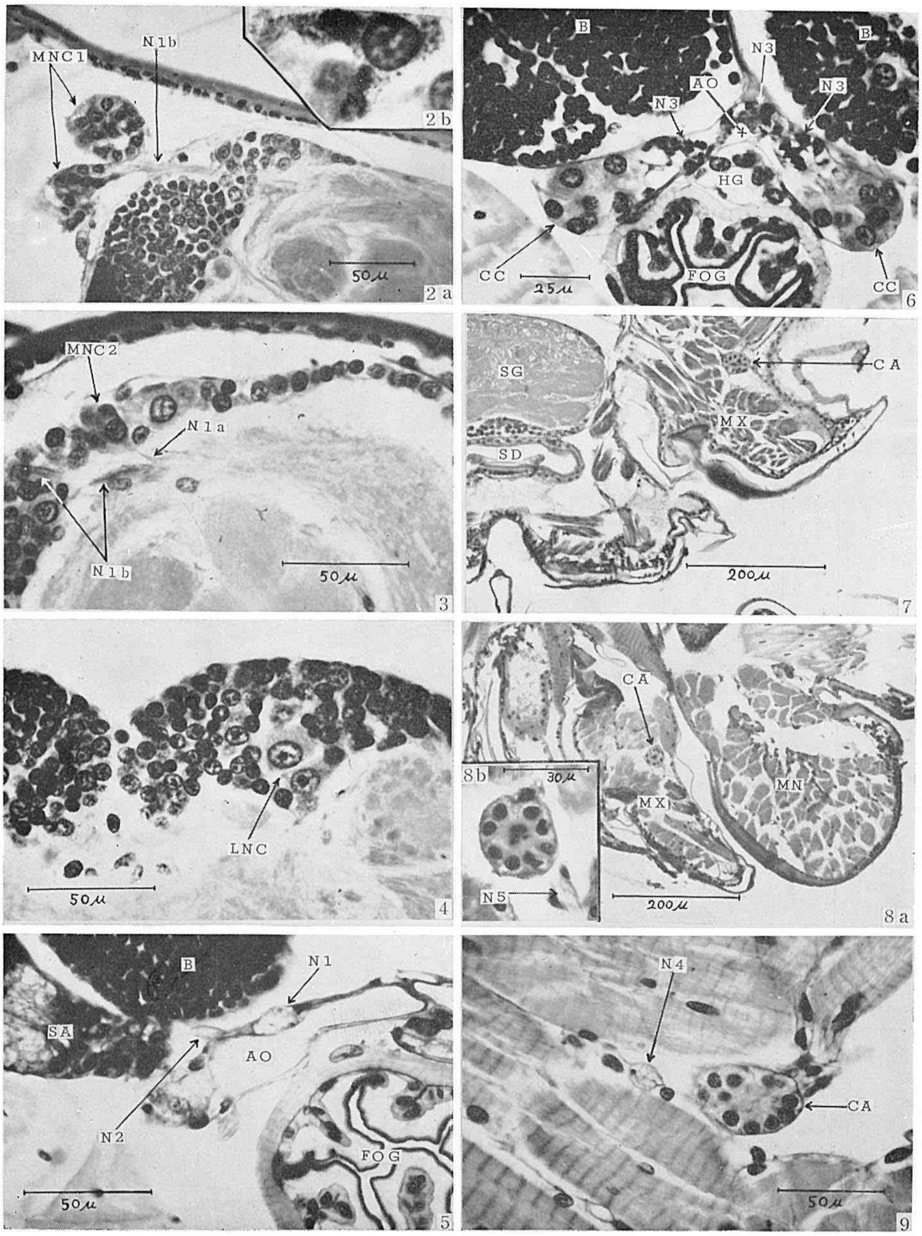
Fig. 7. Transverse section of the head showing the position of the corpus allatum.

CA, corpus allatum; MX, maxilla; SG, suboesophageal ganglion; SD, duct of the ventral salivary gland.

Fig. 8a. Longitudinal section of the head showing the situation of the corpus allatum.
CA, corpus allatum; MN, mandible; MX, maxilla.

Fig. 8b. Enlargement of the corpus allatum with stainable colloid in the central cavity.
N5, upper branch of maxillary nerve innervating the corpus allatum.

Fig. 9. Transverse section of the head through the corpus allatum showing a droplet in nervus corporis allati. This is an exceptional case in which a blue droplet is observed in N4.
CA, corpus allatum; N4, nervus corporis allati.


MNC1
N1b
2b
50μ
2a
B
N3
AO
N3
B
HG
CC
25μ
FOG
CC
6
MNC2
N1a
N1b
50μ
3
SG
SD
CA
MX
200μ
7
LNC
50μ
4
CA
8b
30μ
MX
MN
N5
200μ
8a
B
N1
SA
AO
N2
FOG
50μ
5
N4
CA
50μ
9