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Preliminary Micro-anatomical Study of the Silkworm Larva. I. Nervous System

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การศึกษาจุลกายวิภาคของตัวไหมเบื้องต้น : 1. ระบบประสาท

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** *ผู้อำนวยการสถานีทดลองหม่อน-ไหม ขอนแก่น*

เราได้ทำการศึกษาตัวไหมวัยสามด้วยวิธีการทางจุลกายวิภาคศาสตร์แบบที่เรียกว่าพาราฟินเทคนิค แล้วส่องดูด้วยกล้องจุลทรรศน์ เราได้ศึกษาอวัยวะทุกระบบ ในรายงานนี้เราจะเสนอแต่ระบบประสาทเพื่อเป็นการเริ่มต้น กายวิภาคศาสตร์ของสมองนั้นเป็นแบบที่พัฒนาขึ้นมาพอที่จะเรียกว่าสมองได้ แต่ยังถือว่ายังเจริญทางวิวัฒนาการยังไม่ถึงขั้นที่เราคุ้นเคยกัน ระบบประสาทของมันนั้นส่วนที่เลยจากสมองไปทางหางจะเทียบได้กับ sympathetic chain ของพวกเลี้ยงลูกด้วยน้ำนมเท่านั้น สายโซ่ประสาทอันนี้จะอยู่ในแนวกลางตัวตามยาว และอยู่ถัดจากระบบย่อยอาหารไปทางด้านท้อง ระบบย่อยอาหารเป็นแบบท่อตรง ๆ สายโซ่ประสาทของตัวไหมวัยสามนี้จะเริ่มต้นจาก pharyngeal ganglion ไปสุดที่ลำตัวปล้องที่สิบ ซึ่งเป็นปล้องสุดท้าย สมองของมันอยู่ dorsal ต่อ pharynx และอยู่ในบริเวณหัว ประกอบด้วยสองซีก มี white matter อยู่ข้างใน และมี gray matter อยู่ข้างนอก อันนี้จะเหมือนกับสมองของสัตว์พวกเลี้ยงลูกด้วยน้ำนม สำหรับ white matter นั้นจะติดต่อกันเหมือนกับรูป chiasma สมองแต่ละซีกเป็นรูปสามเหลี่ยมในหน้าตัดขวาง สำหรับสายโซ่ประสาทนั้นจะมี 2 สาย แต่เข้ามาชิดติดกันใน ganglia ที่อยู่ในปล้องลำตัว เรามีแผนที่จะศึกษาสมองให้ละเอียดขึ้นไปอีก

Abstract

The preliminary light microscopic studies of the third instar larvae of the silkworm were carried out by using modified paraffin technique. All organ systems were carefully scrutinized under the light microscope. We are presenting its nervous system to begin with. The brain structure is fully qualified for the terminology but in a less developed state. Its nervous system caudal to the head is at its best comparable to the mammalian sympathetic chains placed in the midventral line under the straight G-1 tract beginning from the pharyngeal ganglion to the last one in the tenth body segment. The brain which lies in the head dorsal to the pharynx is made up of two cerebral halves organized in the fashion shared by mammalian brain, gray matter outside and white matter inside where they communicate in a chiasmatic morphology. The brain is triangular shape in cross section. Further studies of the brain are planned. There are two nerve chains running side by side but coming together at the ganglia.

We found that there are nine ganglia after the pharyngeal one distributed rather evenly about 684 micrometers apart on the nerve chains. The two nerve chains join in each glanglion. The ganglia are invested by the sheath comparable to the dural sheath of human dorsal root ganglion. The silkworm ganglion is about 34 sections of 6 micrometers long (214 μm). The micro-anatomy of each ganglion repeats that of the brain in its organization contrasting the pattern found in the mammalian spinal cord. The cell details down to the ultra-structural level of the whole system must await further studies. Our technique is yet to be improved, we found only few motor-end-plates at the end of the main nerve branch entering the muscle just a little distance off the ganglion.

To establish the firm ground for applied researches on the *Bombyx mori* which is believed to be a remedy for the northeast Thailand farmer's economy, we stepwisely proposed to : 1) improve the study technologies, 2) conduct histochemical studies, 3) study micro-anatomy of all 5 instars to cover the development of the reproductive system which will provide basic informations leading to the study of its molecular biology paving the way to genetic engineering necessary to manipulate this insect at our will, and 4) electron microscopic studies to find the ultimate anatomical background at the exact molecular level

Background

The silkworm has been the biological model for research since medieval times (Tazima, 1978). Besides its great economic values, its morphology is suitable for laboratory use in many fields not less than the fruit fly *(Drosophila melanogaster)* in Genetics. But its detailed anatomy down to the microscopic and electron microscopic levels are not readily available. In the practice of silkworm rearing one requires knowledges in the normal anatomy which lead to the understanding of normal physiology. This, in turn, leads to the insight into the pathology which is actually an abnormal structures and functions. With all these informations it may be possible to manage the abnormalities so that the silkworms live to produce and reproduce effectively better. The advance of new instruments and methodologies also prompt the re-investigation of the silkworm anatomy. The studies of the silkworm so far have been on the biochemical and genetical aspects. It is of a great value to study by the histochemical method of anatomy to see where the actions take place at the molecular level of the structure. We planned to study all organ system but chose to look at the central nervous system first to see its evolution stage to pave the way for relating to its normal function.

Material & Method

The 3rd instar **Bombyx mori** larvae were fixed and processed througth the H&E technique. The serial sections cut at 6 micrometers were examined under the light microscope.

Results & Discussion

The serial sections of the 3rd instar larvae were examined. We observed the central nervous system and found that the system itself, except the brain, is only comparable to the sympathetic chains of mammalians. The two chains come together to lie in the midventral position just ventral to the straight digestive tract. Though we have not studied the system thoroughly because of the still-to-improve techiques, we expected its similarity to that of the bees. The brain lies just dorsal to the anterior end of the pharynx. It consists of 2 symmetrical halves of triangular shape in cross section. The arrangement of the perikarya is enclosing the white matter, a situation found in mammals (Fig 1). How many cell types are there in the brain? What are their spatial relationships? We have no answers now and we planned to do the studies. The white matter appeared to cross like what is observed in the mammalian optic chiasma. From each half of the brain we observed a nerve cord running ventromedially to the subpharyngeal ganglion (Fig 2). And herefrom the rest of the central nervous system lies ventral to the gut ending in the tenth body segment. The micro-anatomy of the ganglia observable and allowed by this still-to-improve technique repeats that of the brain in general (Fig 3). The cell details must be worked out.

We found that there are nine ganglia after the subpharyngeal one distributed rather evenly about 684 micrometers apart on the two nerve chains. The chains separates between the ganglia (Fig 4) and join in the ganglia (Fig 3). There is a sheath covering each ganglion similar to the dura of the mammals (Fig 5). The ganglion is 34 sections of 6 micrometers long (214 micrometers) in average.

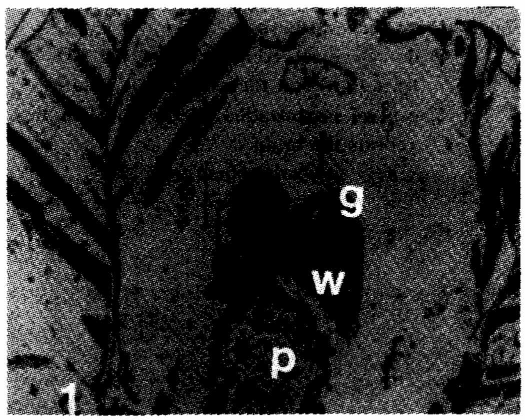

Fig. 1 Cross section of the head of Bombyx mori showing parts of the brain
g = gray matter p = pharynx
W = white matter

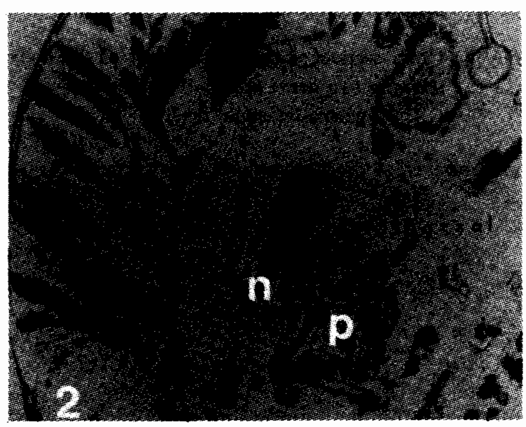

Fig. 2 Cross section caudal to fig.1 showing nerve cord running ventromedially from one half of the brain.
n = nerve cord from one side
p =pharynx

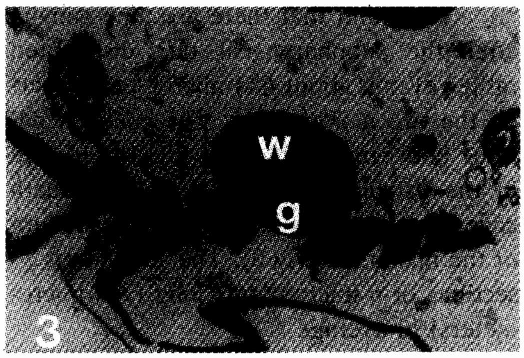

Fig. 3 Cross section showing
1) dorso-ventral relationship of the G-I tract (g.i.) and the ganglion (w.g.)
2) general organization of the ganglion that repeats the brain.
g.i. = gastro-intestinal tract
w = white matter
g = gray matter

Fig. 4 Cross section of the ventral part of the body showing two nerve chains (black arrows)
G.I. = gastro-intestinal tract

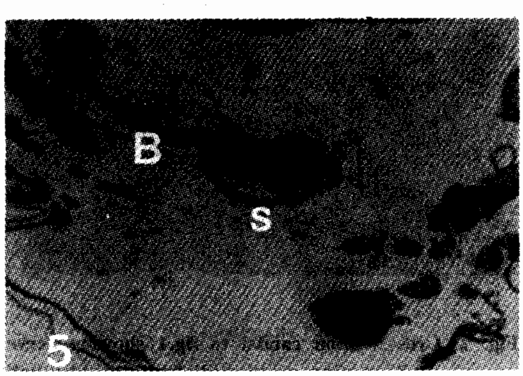

Fig. 5 Cross section of ventral part of the body showing sheath of the ganglion (S), and a nerve branch (B) to the silkgland.

A brief survey of the evolution of the central nervous system is essential for the appreciation of the CNS of **Bombyx mori.** The most primitive nervous system is recorded in the single cell animal in the water. This paramecium possesses a motor center near the gullet; its motor function and sensory function all performed by its specialized protoplasm. The multicellular animals in the water such as hydra developed a nerve net in the middle layer of its body wall. The nerve cell bodies (perikarya) localized among its ectodermal and endodermal cells. In this nerve net appears the first synapse of the nervous system but a two-way synapse. The next advanced brain model is found in the flat worms (Planaria). It has a symmetrical body with a real mesoderm which evolved muscle cells. The first central nervous system evolved in this animal. This first CNS is like a ladder with 2 lobes of brain (or head ganglion) with two nerve cords running to meet near the caudal end. There are nerve fibers forming cross bars at regular intervals completed the structure of a ladder. This CNS lies ventral to the G-1 tract and also possesses nerve nets. The brain here functions as a sensory relay center only. The next more advanced CNS is that of the earthworm. It has a brain as a sensory relay center. Its skin is sensitive to light, touch, and chemicals. The newly evolved structures of the CNS are : 1) subpharyngeal ganglion which could act as a sensory relay center, 2) each ganglion develops a simple reflex arc for the first time, and 3) intersegmental reflex arc between ganglia developed for the first time in this animal. This is regarded as a neural basis of the spinal cord activities in man. The honey bees (Arthopod) organized their body segments into head, thorax and abdomen. The muscles are more developed than just the circular and longitudinal layers of the earthworm. This insect has single eye and compound eye to see and the antennae to detect light and smell in the head regions. It must fly near and far to collect the nectar. It needs all necessary CNS structures to perform all the aforementioned functions. Its brain can no longer function only as sensory relay center but must do all kinds of motor functions. Its social behaviors are also controlled by the CNS. The brain of this insect, therefore, is the most developed of the invertebrates. The silkworm, **Bombyx mori,** attains the adult stage which do not fly but moves the wings very fast while mating and walking to find the mating partner. We would be able to conclude after the complete studies that the silkworm CNS is to a certain degree similar to that of the honey bees.

We found nerve branches to the muscle (Fig 6) and probably to the silk gland (Fig 5). The detail of the neuromuscular structures have to await further studies. Since the animal has been totally domesticated from the 10th century, its life activities are not more than moving to find mulberry leaves and to build the cocoons. It is interesting to study the motor-end-plate both its anatomy and physiology since this animal is the member of the class (insecta) who first developed the neuromuscular mechanism that higher forms inherited.

This study is only the beginning and only little observation on general morphology is presented. The next studies will be on the detail of the central nervous system and its derivatives.

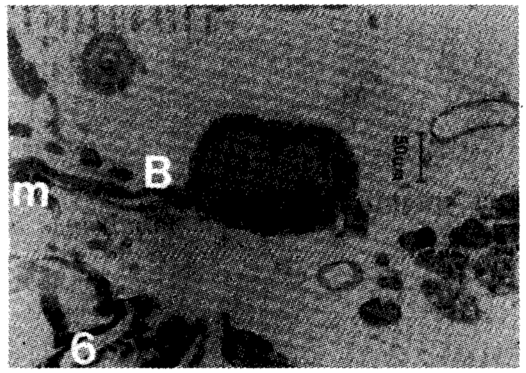

Fig. 6 Cross section of the ventral part of the body showing a nerve branch (B) leaving the ganglion for the muscle (m)

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