

The present study has been undertaken in the hope of observing the internal architecture of the silk gland nucleus, as well as demonstrating polytene chromosomes in acetic orcein squashed preparations.

The silk glands were dissected out from larvae at various developmental stages. They were soaked in a dissociating solution which is a 9:1:4 mixture of 0.5% acetic acid, glycerine and distilled water. After several minutes, the gland cells begin to be detached, and finally they are dispersed in the solution. The cells were picked up by a fine glass pipette and placed on a slide glass. After removal of the excess dissociating solution, the cells were squashed with acetic orcein.

Until the third day of the third instar, the nuclei are rectangular in form. At this stage, thick and distinct strands, about 0.5μ in diameter, are seen running nearly parallel to the long axis of the nucleus, being about 60 in number which approximately corresponds to the diploid chromosome number of 56 (Figures 1 and 2). The strands are twisted at many loci, showing differential coils along their length so that the nucleus appears to have a banding pattern. It has not been established that the position of the differential coils and their number are constant for a given strand. The nucleus of the second day of the third instar is about 200μ in length and 20μ in width. Thus the strand is at least several hundred times as long as that of the metaphase chromosome observed in a spermatogonium (Figure 3).

After the third day of the third instar, the nucleus begins to show a characteristic transformation, resulting in the formation of the ramified nucleus. At the end of the larval development, the ramified nucleus occupies the whole cell. A preliminary study by Feulgen-microspectrophotometry on a relative amount of DNA in the ramified nucleus of the gland cell on the sixth day of the fifth instar larva (18 days after hatching) has given the

value of about 200,000 times as much as that of the diploid nucleus. This value strongly suggests that DNA replication has occurred at least 17 or 18 times during the growth of the silk gland.

Though any final conclusion should be left for future studies, it seems most probable in the light of the findings described above that the long 0.5μ strands observed in the nucleus may be produced by repeated endomitoses, and they may be no other than polytene chromosomes. Further quantitative studies on the structure and function of the polytene chromosomes, as well as on the mechanism of the ramification of the nucleus are now in progress by means of various techniques such as electron microscopy, autoradiography and microspectrophotometry.

Zusammenfassung. Es werden bei *Bombyx mori* ca. 60 signifikante Bänder von $0,5 \mu\text{m}$ Durchmesser, offenbar als polytane Chromosomen, parallel der Achse der Spinn-drüsenkerne mit verschiedenen Methoden sichtbar gemacht.

Y. H. NAKANISHI, H. KATO
and S. UTSUMI⁴

National Institute of Radiological Sciences,
Chiba (Japan), 21 October 1968.

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⁴ Permanent address: Botanical Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

The Occurrence of Bipolar Neurons in the Abdominal Mass Ganglia of a Pulmonate Mollusc (*Cryptomphallus aspersa*)

Numerous anatomical and electrophysiological investigations have been made in the last few years on giant neurons of molluscs, specially in gastropods, such as *Aplysia*¹⁻³ and *Cryptomphallus aspersa*^{4,5}. These elementary systems have proved to be suitable models to analyse basic problems of neurobiology at the cellular level.

In most studies these neurones have been considered to be unipolar and only in a few cases have bipolar neurons in the cerebroid ganglia been reported⁶. In the present study the existence of several types of bipolar neurons in the abdominal mass ganglia of the land snail *C. aspersa* will be reported.

The abdominal mass ganglia of 30 adult specimens were used. The entire nervous ring (which includes the abdominal mass and the cerebroid ganglia) were excised, pinned on a cork sheet and plunged into warmed (at 37°C) Bouin fixative for 48 h. They were then dehydrated, cleared and embedded in Paraplast. Frontal sections cut at 8μ were stained with GABE's technique for neurosecretory material⁷.

Most neurons appear to be unipolar and with GABE technique show neurosecretory granules around the nucleus or in the axon hillock (Figure 1). However, besides

this more common type, bipolar cells having processes of different type were recognized. One type of bipolar neuron (Figure 2) shows both processes near one another. In the perikaryon, there are 2 clusters of neurosecretory material near each axon hillock. Other neurons have both processes originating at the same point of the perikaryon (Figure 3). Finally there are few ones which show the 2 processes coming from opposite sites (Figure 4). In this last type, corresponding to a medium-size neuron, the neurosecretory material appears only in the axon hillock of one process.

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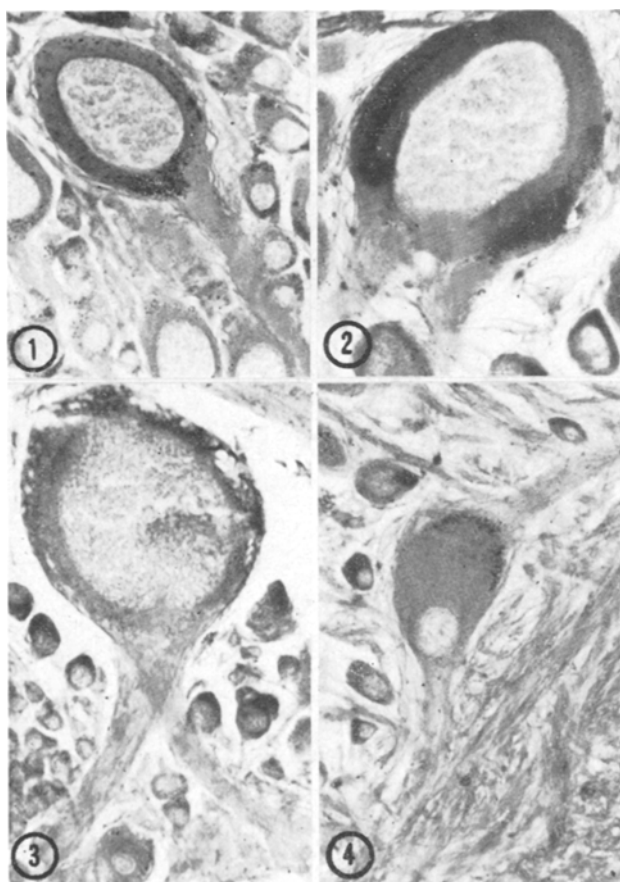


Fig. 1. A typical monopolar giant neuron of the abdominal mass ganglia of land snail *Cryptomphallus aspersa*. Small neurosecretory granules appear in the axon hillock. $\times 1090$.

Fig. 2. A bipolar neuron with both processes near one another. Observe the presence of 2 clusters of neurosecretory granules near each axon hillock. $\times 1090$.

Fig. 3. Another bipolar neuron with both processes originating at the same point of the perikaryon. $\times 1090$.

Fig. 4. A medium-size neuron with 2 processes coming from opposite sites. Here neurosecretory material appears in one axon hillock. $\times 1090$.

As mentioned above, these bipolar neurons are far less numerous than the unipolar ones.

TAUC⁸ described in the giant neurons of *Aplysia* an intermediary segment, where the action potential originates. In this case, the soma of the giant neuron can be removed, and the activation of the intermediary segment still produces the firing of all the axonic processes. Besides this type of neuron, TAUC and HUGES⁸ have described another one in which the intermediary activator segment does not exist. These are cells in which the activation of an axonal branch cannot invade other branches without a previous somatic action potential.

It may be assumed that the bipolar neurons described here (Figures 2, 3 and 4) correspond to the last neuronal group proposed by TAUC and HUGES. It may be postulated that in such bipolar neurons each axon acts as an isolated unit, and that the activation of the perikaryon is the condition needed for the synchronic function of both axonal processes⁹.

Resumen. Se describen en la masa ganglionar ventral del molusco *Cryptomphallus aspersa* (Gasteropoda, Pulmonata) la presencia de varios tipos de neuronas bipolares entremezcladas con las típicas unipolares. Estas observaciones morfológicas se discuten en relación a los hallazgos obtenidos previamente con técnicas electrofisiológicas por otros autores.

C. A. SANCHÍS and D. ZAMBRANO

*Instituto de Anatomía General y Embriología,
Facultad de Medicina y Cátedra de Histología,
Facultad de Farmacia y Bioquímica,
Buenos Aires (Argentina), 21 November 1968.*

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Centromere Nature of the Chromosomes of *Ranatra* (Heteroptera)

The chromosomes of the heteropteran insects were considered for long to possess a 'diffused' type of centromere¹⁻⁵. PRASHAD⁶, however, disagreed with this view. He regards the centromere of the chromosomes in Lygaeidae and Coreidae, to be neither of the diffused type nor of the typical localized 'monocentric' type. According to him, this 'atypical' centromere in these 2 families does not impair the abilities of the chromosomes to incorporate themselves into the spindle even though they undergo fragmentation. Hence despite repeated fragmentations, the broken chromosomes do not disappear from the populations. Therefore the 'diffused' or 'atypical' centromere nature of these chromosomes attains a special significance as it has much bearing upon the origin of

multiple sex chromosomes and the supernumerary chromosomes in these insects. However, our observations on the chromosomes of another heteropteran insect, *Ranatra elongata* are not in agreement with either of the above-mentioned views.

R. elongata has extremely short and squarish chromosomes. The males have a diploid number, $2n$ as 43 consisting of 38 autosomes and 4 X and 1 Y (Figure 1). A careful observation reveals that these chromosomes are not merely irregular dot-like or oval bodies as shown in the works of DAS¹, and STEOPE⁴, but have definite configurations. They are squarish in outline, each with a distinct constriction in the middle or so. Configurations of the early metaphase I chromosomes are of special