small nodules comprising only a part of the callus and usually lay at the defect's margin on the dural side of the bone. The cartilage was then in close proximity to brain tissue herniating through the defect (Figure 1). A χ^2 test shows the difference in the occurrences of cartilage between deficient and supplemented groups to be significant at the 0.1% level. Moreover, the cartilage formed in the one vitamin A-supplemented rat was extremely scanty (Figure 2).

Most deficient rats did not eat and lost weight postoperatively. Although their deficiency thus was probably multiple, adequate materials were recruited to form a bony callus which included the islands of cartilage.

Possible factors promoting the development of this cranial cartilage could be: (1) an ischaemia[8] resulting from a pressure by the herniating brain; (2) a mechanical action by the mis-sized brain[9] on osteoprogenitor cells of the callus; (3) a chemical inductive effect by the brain tissue[10]; (4) a regressive return to the cartilage-forming tendencies of neonatal development at sutures[11] or neonatal repair[4]; or (5) a direct action on the callus-forming cells of the low level of vitamin A. Excessively high levels of vitamin A cause cartilage destruction in vitro[12]; low levels in vivo might provide a permissive environment in which cartilage could be formed and survive[13].

Zusammenfassung. Die Heilungsvorgänge bei experimentell erzeugten Defekten der Parietalknochen junger Ratten wurden untersucht: Knorpel entwickelt sich im Kallus nach 9–28 Tagen bei Tieren mit einem Vitamin-A-Defizit, jedoch nur in einem von 19 Kontrolltieren, welche Vitamin A zusätzlich erhalten haben.

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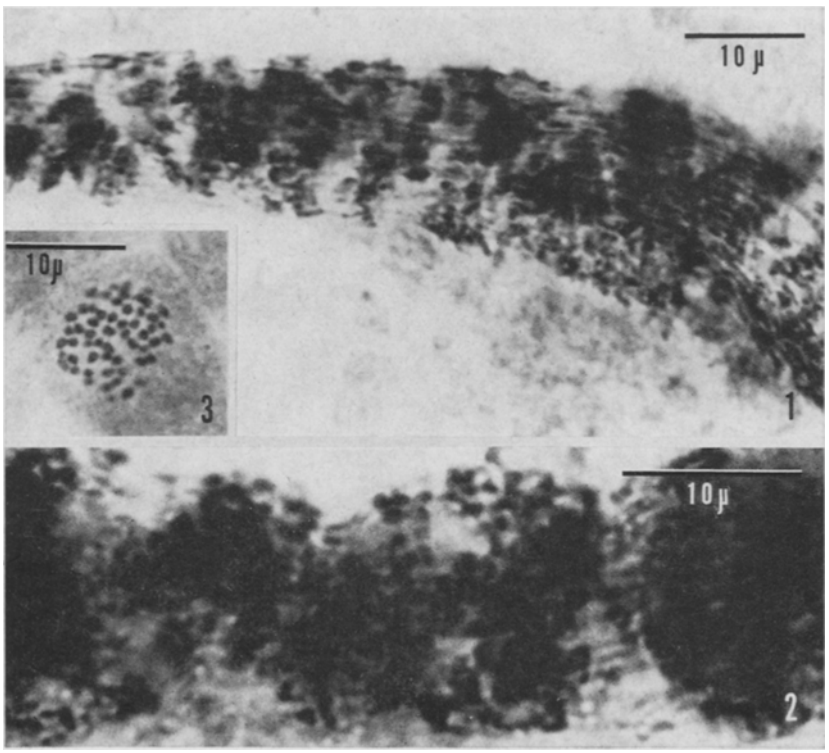
Polytene Chromosomes in Silk Gland Cells of the Silkworm, *Bombyx mori*

It is well-known that the silk gland of the silkworm becomes bigger without increase in the number of cells, or without division of cells, in the advance of growth after hatching[1,2]. On the basis of the results obtained by electron microscopic autoradiography, Akai and Kobayashi[3] have suggested that a function of the chromatin in the nucleus of the silk gland is like that of the polytene chromosomes in the salivary gland of Diptera. However, the polytene chromosomes in the silk gland nucleus have not been observed by them. So far as we are aware, no adequate literature pertaining to this subject is accessible.



Figs. 1 and 2. Thick and distinct strands are seen running nearly parallel to the long axis of the silk gland nucleus of the silkworm. The strands, presumably polytene chromosomes, are twisted at many loci, showing differential coils along their length. Fig. 3. Metaphase chromosomes in a spermatogonium of the silkworm.

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 0.5 μ 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 200 μ in length and 20 μ 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 0.5 μ 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 μm Durchmesser, offenbar als polytäne Chromosomen, parallel der Achse der Spinndrüsenkerne mit verschiedenen Methoden sichtbar gemacht.

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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 reported[6]. 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 8 μ were stained with GABE's technique for neurosecretory material[7].

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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